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SYMBIOTIC SUCCESS: THE IMPACT OF INDIGENOUS RHIZOBIAL STRAINS ON RAINFOREST SOYBEAN PRODUCTION

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Abstract
Mud crabs, belonging to the genus Scylla, are prized edible crustaceans found in the tropical Indo-West Pacific region. They include species like Scylla serrata, Scylla tranquebarica, Scylla olivacea, and Scylla paramamosain, each distinct in its own right. These crabs are not only known for their delicious flavor but also for their nutritional value, boasting essential amino acids, proteins, polyunsaturated fatty acids, and vital minerals like calcium, iron, zinc, potassium, and phosphorus. Mud crab aquaculture is crucial for small-scale fisheries in the Indo-Pacific region, contributing to commercial demand. One method employed is crab fattening, which involves rapidly increasing the crab's weight and size. This process can take anywhere from 15 to 60 days and is carried out in various enclosures, including earthen ponds, bamboo enclosures, net cages, floating cages, and plastic boxes. Successful crab fattening relies on factors such as providing essential crab nutrition, maintaining water flow and quality, monitoring soil quality, and managing crab density within the ponds. Proper attention to these variables ensures that "water crabs" or "empty crabs" can attain the desired size and meet market demand.

Keywords: Mud crabs, Scylla species, crab fattening, aquaculture, nutritional value

Introduction

Soyabean (Glycine max (L.) Merill) is an important legume crop grown in many parts of Nigeria where it is a major source of protein. The importance of soyabean is not limited to dietary benefits but also soil productivity enhancement. Soyabean plays a key role in agricultural systems that help to improve soil fertility in the sub Saharan Africa (Sanginga et al., 2000b). This is achieved through rotation of soyabean with other crops because of its ability to fix atmospheric N into soil through its symbiotic association with rhizobia. Rhizobia are soil free living bacteria infecting the root of leguminous plants and forming nodules which help to fix N in the soil. Despite the free existence of rhizobia in soil, most soyabean production systems prefer inoculation of seeds before planting with rhizobial inoculants that have been proved to be efficient in N-fixation and yield improvement of soyabean. In the case of Nigeria and other parts of Africa where soyabean was not an indigenous crop, inoculation with rhizobial inoculant has always been advised even for the promiscuous varieties (Abaidoo et al., 2007; Giller et al., 2011).

The indigenous rhizobia are capable of infecting plant roots and therefore can play a significant role in symbiotic biological nitrogen fixation and growth and yield of most leguminous crops. Sanginga et al. (2000a) reported that symbiotic performance of indigenous rhizobial strains depends on their population size, survival and effectiveness. The search for indigenous rhizobial strains capable of inducing efficient nodulation in Nigerian soils has been on-going for a while. Ranga-Rao et al. (1984) reported that some indigenous rhizobial strains from Southwestern Nigeria nodulated and fixed atmospheric nitrogen better with Indonesian soyabean cultivar (Orba) than American cultivars TGm

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80 (Bossier) and TGm 294 – 4. Okereke and Onochie (1996) reported poor nitrogen fixing ability of the native Bradyrhizobium species in Nigerian soils when screening the native and foreign Bradyrhizobium japonicum strains for high nitrogen fixation in soyabean. Okogun and Sanginga (2003) reported that tropical soil rhizobial strains were not effective in fixing enough biological nitrogen to sustain soybean growth and thus, the need for a starter dose of nitrogen fertilizer or a search for more effective rhizobial strains.

Selection for effective rhizobia for N fixation in soyabean should include processes that screen indigenous rhizobia and it may take considerable time to achieve. Soybean inoculation and N fixation was successful in Brazil due to selection program that took place in over half a century (Hungria et al., 2006; Hungria and Mendes, 2015). Selection should also be tailored to a specific context based on the evidence that biological N fixation is affected by the source of inoculant, crop varieties, management practices across countries, soil and climatic conditions (Ronner et al., 2016; Hardadson, 1993). Studies on indigenous rhizobia for N fixing efficiency in soyabean in the forest transition zone of Nigeria are at best scanty. Ojo et al. (2015) selected three indigenous rhizobial strains that are highly infective on soybean in the forest transition zone of Nigeria but information on their N fixation efficacy was lacking. Forest transition zone of Nigeria is generally known for its heavy rainfall, high relative humidity, aggressive vegetation growth and high organic matter. This zone is characterized by smallholder and low input agriculture. The practice of seed inoculation before planting is a challenge to most local soyabean farmers because of the scarcity and cost of purchasing exotic inoculants. To increase soyabean inoculation and N fixation in the zone, it is imperative to search for efficient indigenous rhizobial strains in the soil that could fix N better than or comparable to the available exotic strains. These effective indigenous rhizobia can be produced as cheap inoculants to farmers to improve and harness the benefits of biological nitrogen fixation for sustainable agricultural production in Nigeria. Therefore, the aim of the study was to evaluate the performance of three indigenous rhizobial isolates for N fixation in soybean in the forest transition zone of Nigeria.

☐ Methods

☐ Location, climates and soils of the experimental sites

Field trials were conducted in three different locations namely: Idi-Ayunre (IA), Orile-Ilugun (OI) and the University of Ibadan Teaching and Research Farm (UITRF) within the rainforest-savanna transition zone of Nigeria. Soils from the three locations were also collected and used for the greenhouse study. Idi-Ayunre in Oluyole Local Government area of Oyo State lies within latitude 7°26´N and longitude 3°54´E. Idi Ayunre's soil is derived from metamorphic basement complex rocks and it is classified as Nitosol (USDA, 2006). The soil is predominantly Olorunda Soil series (Smyth and Montgomery, 1962). Orile-Ilugun in Odeda Local Government area of Ogun State lies within latitude 7°13´N and longitude 3°31´E. The soil belongs to Apomu Soil series (Smyth and Montgomery, 1962), derived from metamorphic rock and classified as Luvisols (USDA, 2006). The UITRF is within latitude 7°30´N and longitude 3°45´. The soil of UITRF is classified as Alfisols (USDA, 2006) and it belongs to Egbeda soil series which is derived from basement complex of rocks granite (Smyth and Montegomery 1962). None of the fields had been cultivated to cowpea or soyabean in the last ten years nor had history of rhizobial inoculation.

Soil samples were analysed for pH in water (1:1) as outlined in IITA (1982), soil organic matter using wet dichromate acid oxidation method (Nelson and Sommers, 1982), total nitrogen using Kjeldahl analytical method (Bremmer and Mulvaney, 1982), available phosphorus using Bray-1 method (Bray and Kurtz, 1945), particle size using Bouyoucus hydrometer method (Okalebo et al., 1993), exchangeable Mg, Ca, K and Na extracted using neutral 1M ammonium acetate and determined with spectrophotometer (Okalebo et al., 1993). The rhizobial population count of the three locations was

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determined as described Ojo et al. (2015). The soil biological, chemical and physical characteristics of the three locations are presented in Table 1.

☐ **Pot experiments**

Three separate experiments were conducted to determine nodulation in three soyabean varieties using topsoils (0-15 cm) that were collected from the three selected locations for field experiments, UITRF, OI and IA.. The soils were sterilized using direct flaming method with the aid of a Terraforce sterilizing machine (IITA fabricated sterilizing machine), air-dried and sieved with 2 mm sieve. Two kilograms soil was weighed into each pot for planting. Each experiment was a factorial combination of soil sterilization, sterile and non-sterile; three varieties of soyabean, TGx 1448–2E, TGx 1908–1F, TGx 1910–2F and six rhizobial inoculation treatments, control, OISa-6e, IDC8, TRC2, R25B and IRj2180A, laid in completely randomized design. Rhizobial strains IDC8, OISa-6e and TRC2 were highly infective indigenous rhizobia isolated from the soils of locations used in the field trials (Ojo et al., 2015). Rhizobial strain R25B and IRj2180A, used as reference strains, were collected from IITA and termed exotic in this study. Each treatment was replicated three times. A total of 108 pots were planted per experiment or location

Four seeds were planted per pot and thinned to two plants per pot at five days after planting (DAP). Inoculation was done one week after planting using direct broth culture inoculation method (Somasegaran and Hoben, 1994). With the aid of dispenser, the two plants in each pot were inoculated with 2 ml Yeast Mannitol Broth (YMB) culture of each rhizobial strain. Pots were water with distilled water on daily basis.

At 8 weeks after planting (WAP), the shoots of the plants were cut at the soil level and weighed for fresh shoot weight/pot. The leaves and petioles were detached from the stems. The samples were air-dried for 72 hours and oven-dried at $78^{o}C$ to constant dry weights. Soils were washed off the roots and nodules on the roots and those detached were picked and counted together and expressed in number/pot. Fresh weights of nodules and roots were determined. Dry weights of nodules were determined by oven-drying at 78oC to constant weights.

Table 1. Soil properties of the study sites

Soil properties	Idi Ayunre	Orile Ilugun	UITRF
pH (KCl)	6.55	6.09	5.76
Total N (g/kg)	0.23	0.17	0.08
Available P (mg/kg)	0.42	0.06	0.13
Ca (cmol/kg)	7.87	7.48	4.84
Mg (cmol/kg)	2.59	1.88	1.65
K (cmol/kg)	0.83	0.67	0.85
Na (cmol/kg)	0.44	0.39	0.43

Fe (mg/kg)	25.34	31.45	26.29
Mn (mg/kg)	14.74	17.41	12.38
Sand (g/kg)	645.0	675.0	812.5
Clay (g/kg)	185.0	185.0	100.0
Silt (g/kg)	170.0	140.0	87.5
Rhizobial count (cell g^{-1} soil)	3.80	7.81	13.54

- **Field trials**

Field experiments were set up in a two-factorial arrangement with rhizobial strain and soyabean variety implemented in a split block design with three replicates of 4 m × 4 m each. The rhizobial strains were IDC8, TRC2, OISa-6e, R25B+IRj2180A and the control and soybean varieties were TGx 1448-2E, TGx 1908–IF and TGx 1910-2F. Strains R25B and IRj2180A were exotic strains used separately in the greenhouse experiments but combined together for the field experiment. One kilogramme of seeds of each soyabean variety was inoculated with 10 grammes peat culture of each rhizobial strain. The procedure followed was as outlined by Somasegaran and Hoben (1994). The YMB cultures of each strains were aseptically injected into different peat package carriers using manually operated syringes at ratio 1 : 1 (ml/wt in g) broth culture to peat. Inoculated peats were incubated for 2 weeks at 28 ^{o}C to gain excess of 10^8 - 10^9 cells g^{-1}. Fifty peat-inoculated seeds were randomly sampled from each legume planted and kept in a cool container for enumeration of viable rhizobia on the seeds in the laboratory using spread plate method of estimation as outlined by Somasegaran and Hoben (1994). Planting was done at the three locations between July and early August, being the beginning of the second growing season, and the recommended planting period for soybean in the rainforest zone of Nigeria.

Soyabean was planted 4 - 5 cm deep by drilling and thinned five DAP to 75 cm × 5 cm 1 plant / stand, to give a total population of 266,666 plants/hectare. Weed control was done manually using hoe.

1) Data collection

- **Nodules**

Plants were uprooted systematically at three points per plot using a 30 cm × 30 cm quadrant. Root of five plants and soil under the quadrant area were removed to a depth of 15 cm to determine number of nodules. The nodules on the root and those detached in the soil were counted and weighed to get the fresh nodule weight and oven-dried at 78^{o}C to a constant dry weight.

2.4.2. Sampling for stem + petioles tissue extraction for ureide - N analysis

The leaves of the five sampled plants per plot were detached fresh from stem + petioles. The stem + petioles were weighed fresh, air-dried for 72 hours, oven dried at 78^{o}C to constant dry weights and ground. Subsamples were taken for tissue extraction (Hot water extract) for Ureide – N and NO_3 – N analyses in the laboratory. The procedure followed was as outlined by Herridge (1982). Sub-samples were also taken from leaves, stem + petioles for shoot N analyses in the laboratory using the method of Herridge (1982). The relative ureide-N (RU) abundance of the samples was calculated based on the molar concentrations of ureides and nitrate and RU was used to determine the plant N derived from fixation (described as % Nitrogen Derived from Atmosphere in this study) according to Herridge et al. (1990). Harvesting was done at physiological maturity of each variety. Dry pods were threshed,

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winnowed and sun-dried to obtain field grain yield. Fifty grains of each soyabean variety planted were taken for % N in the laboratory.

1) Statistical analysis

Data were checked for normality of errors and homogeneity of variances before analysis. Number of nodules and NDFA (%) were transformed using logarithm to base 10 and square root transformation, respectively. Data were subjected to Analysis of variance (ANOVA) using PROC GLM of Statistical Analysis System (SAS, 2003). Means of main effects were separated using Least Significance Difference (LSD) at $P<0.05$. Standard Errors of Mean was reported for interaction effect.

1. Results

1) Pot experiments

The nodule formation in UITRF soil was significantly ($P<0.05$) reduced in sterile soil compared to nonsterile soil (Fig.1). Nodulation in the uninoculated plants was also significantly ($P<0.05$) reduced when compared to the inoculated plants. The exotic strain R25B had the highest number of nodules in all the three varieties under non-sterile soil and sterile soil and these numbers were more than twice higher than uninoculated plants. Nodulation in IA soil was very low, less than 10 nodules per pot, and more nodules were significantly ($P<0.05$) formed in sterile soil than in non-sterile soil unlike UITRF and OI soils (Fig. 1). Soyabean variety TGx1908-1F inoculated with TRC2 in sterile soil produced the highest number of nodules. In non-sterile soil, isolate OIa6(c3a) did not form nodules in any of the varieties. No nodulation was observed in TGx1908-10F inoculated with indigenous strains IDC8 and TGx1910-2F inoculated with R25B. In OI experiment, nodule formation was significantly ($P<0.05$) higher in non-sterile soil compared to sterile soil (Fig. 1). The nodulation of plants in sterile soil was very low except in the strain IRj2180A inoculated plants. In fact, OIa6(c3a) inoculated plants did not form nodules in sterile soil. No nodule was also formed in the sterile control pots.

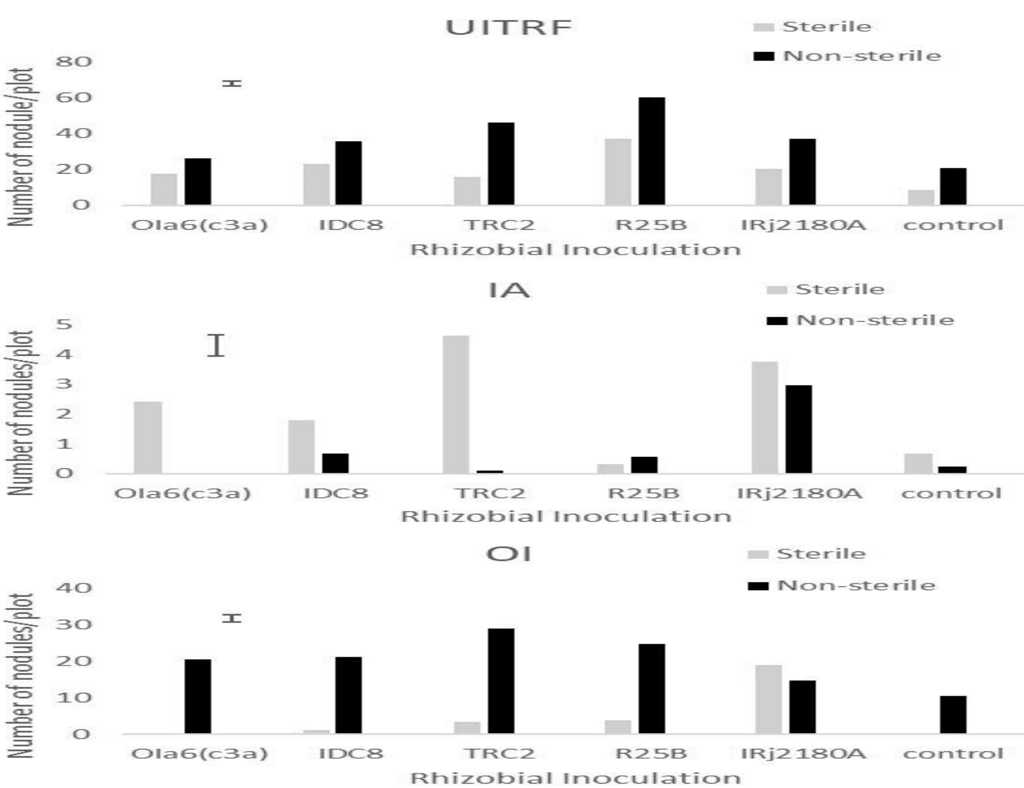

Figure 1. Number of nodules as affected by rhizobial inoculation and soil sterilization on three soyabean varieties in a pot experiment. Bars represent SE of mean

1) Field trials

The analysis of variance for field trials revealed that rhizobial inoculation significantly influenced the number of nodules/plot in each of the study sites (Table 2). While rhizobial inoculation had no effect on all variables in OI except the number of nodules, all the variables were significantly affected by rhizobial inoculation at IA. Variety had no significant effect on all variables tested at IA and UITRF (except number of nodules). Interaction of variety and rhizobial inoculation had significant effect on the grain yield and N uptake of soyabean at UITRF % NDFA and total N fixed at IA and grain N uptake at OI (Table 2)

A) Number of nodules

Soyabean varieties TGx1910-2F and TGx1908-1F formed significantly ($P<0.05$) higher number of nodules than TGx1448-2E at UITRF (Fig. 2). The exotic strains, R25B+IRj2180A, significantly ($P<0.05$) formed more nodules than other strains. Variety TGx1908-1F and TGx1910-2F inoculated with strain R25B+IRj2180A had over 100 nodules more than the other inoculated plants at UITRF. At IA, plant inoculated with R25B+IRj2180A significantly ($P<0.05$) had higher number of nodules than other plants while the uninoculated plants significantly ($P<0.05$) had lower number of nodules compared to other plants, less than 20 nodules for each variety (Fig. 2). Apart from R25B+IRj2180A inoculated plants, other plants with more than 100 nodules were inoculated by strain IDC8. The only effect of rhizobial inoculation at OI was on the number of nodules where R25B+IRj2180A and TRC inoculated plants had significantly ($P<0.05$) higher number of nodules compared to other inoculated and uninoculated plants.

Table 2. Analysis of variance from generalised linear model procedures for variables measured in the field trials, as influenced by variety and rhizobial inoculation in each of the study sites

ns = not significant

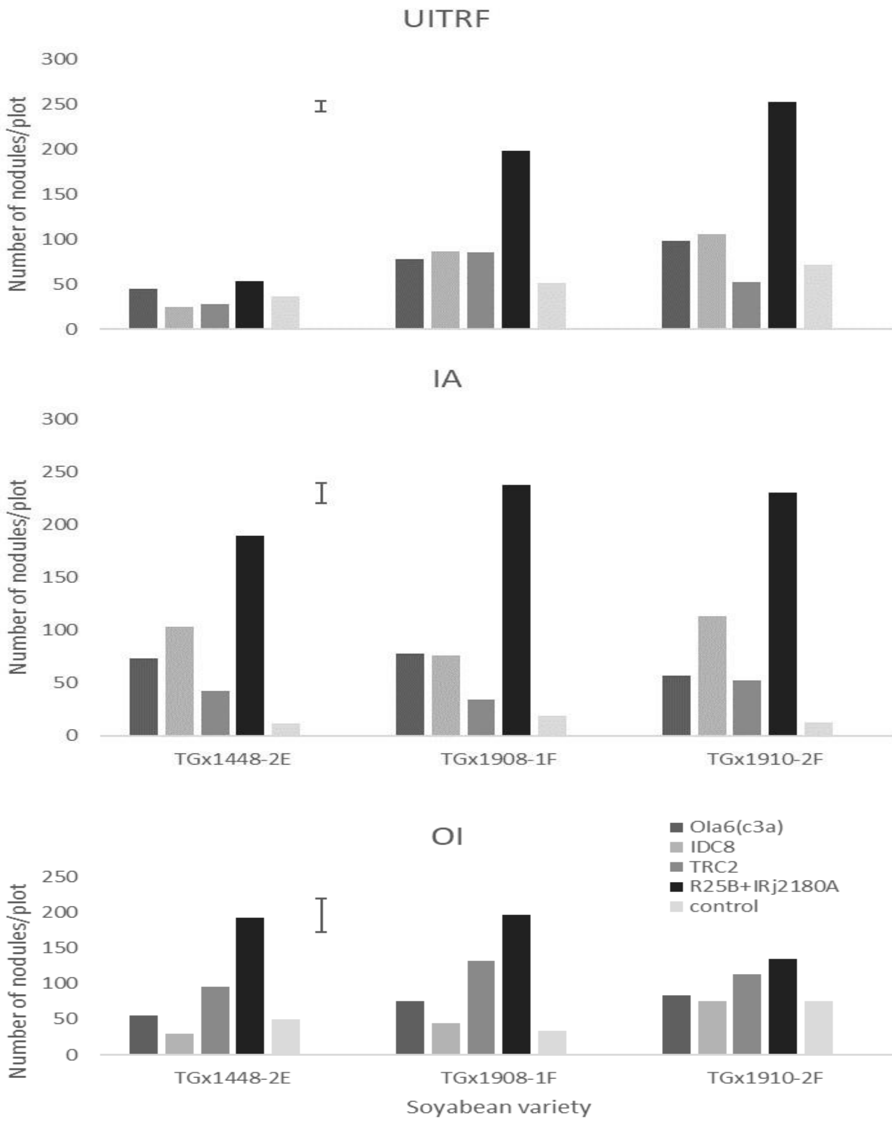

Figure 2. Number of nodules as affected by rhizobial inoculation of three soyabean varieties in the three study sites. Bars represent SE of mean

A. Grain yields and shoot dry weights

The interaction of TGx1908-1F and rhizobial strain R25B+IRj2180A at UITRF led to significantly higher grain yield when compared to other varieties with no inoculation (Table 3). The variety TGx1908-1F inoculated with R25B+IRj2180A is the only treatment with grain yield more than 3 t/ha in UITRF. (Table 3). While the average grain yield for the uninoculated plants was approximately 1.5 t/ha, the average grain yield for plants inoculated with the indigenous isolates was 1.9 t/ha at UITRF. The R25B+IRj2180A inoculated plants had significantly higher grain yield and shoot dry weight than other plants at IA. The average grain yield for R25B+IRj2180A inoculated plants at IA was 3.6 t/ha compared to other inoculated plants, 2.0 t/ha, and uninoculated plants 1.3 t/ha. The highest grain yield (> 4.0 t/ha) in this study was observed in soyabean variety TGx1908-1F inoculated with R25B+IRj2180A at IA (Table 3). The shoot dry weight of OIa6(c3a) inoculated plants was also significantly higher than the uninoculated plants at IA. The grain yield of soyabean ranged between 0.7

– 1.8 t/ha at OI, values that were generally lower to those of UITRF and IA. Grain yield of TGx1908-1F was significantly lower compared to the other varieties. Orile Ilugun was the only study site with grain yield not above 2 t/ha (Table 3).

B. Nitrogen Uptake

The grain N uptake of TGx 1908-1F inoculated with R25B+IRj2180A was significantly higher than those of other treatments and the ratio of accumulation was about 5:1 when compared with the shoot N uptake at UITRF (Fig. 3). Nitrogen uptake was at least more than two-fold higher in the grain compared to the shoot in all plants at UITRF. The shoot N uptake of TGx1910-2F inoculated with TRC2 at UITRF was over 120 kg/ha N and all plants that accumulated above 100 kg/ha N in the shoot at UITRF were inoculated with the indigenous isolates. The grain N uptake in R25B+IRj2180A inoculated plants was significantly higher than other inoculated plants at IA, and their combined grain and shoot N uptake exceeded 500 N kg/ha for each variety (Fig. 3). Also, the shoot N uptake of TGx 1448-2E inoculated with OISa-6e and IDC8 at IA was significantly higher than other plants except R25B+IRj2180A inoculated plants. The grain N uptake was significantly lower in variety TGx 1908-1F compared to the other varieties at OI. Shoot N uptake was not significantly affected by the treatments applied at OI (Fig. 3)

Table 3. Grain yield and shoot dry weight of three soyabean varieties as affected by rhizobial inoculation in three locations

						UITRF			IA			OI
Variety weight (t/ha)	(t/ha)	Strain yield	Shoot dry weight (t/ha)	(t/ha)	Grain yield	Shoot dry weight (t/ha)	(t/ha) 1.0	Grain yield	Shoot dry		Grain	
TGx1448-2E		OIa6(c3a)		3.9	2.0		5.7	1.9		2.7		

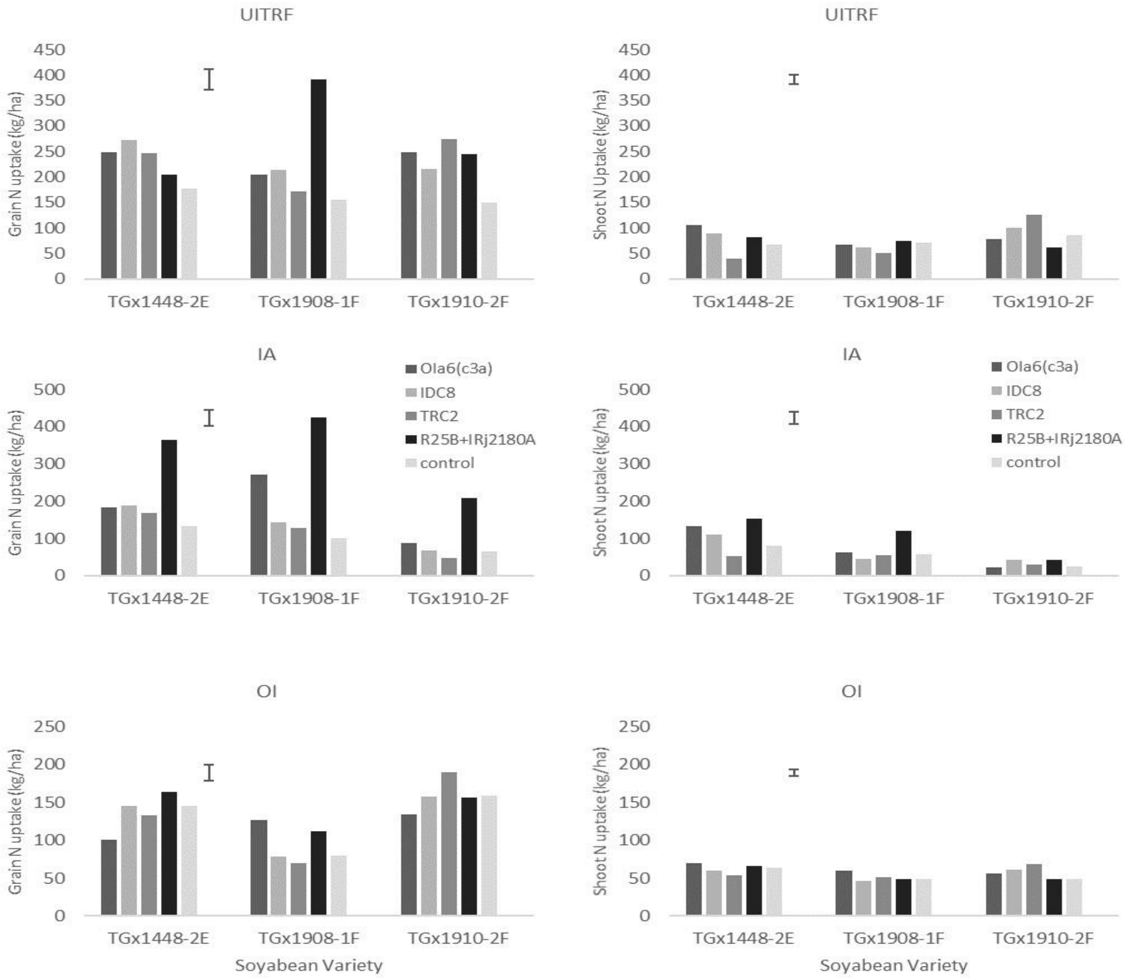

Figure 3. Rhizobial inoculation effect on the grain and shoot N uptake of three soyabean varieties in each of the study sites. Bars represent SE of mean

A) Nitrogen Fixation

The amount of N fixed by the indigenous isolates inoculated plants was comparable to that of R25B+IRj2180A inoculated plants at UITRF. TGx 1448-2E plants inoculated with OISa-6e and IDC8 had above 200 kg/ha N fixed (Fig. 4). However, indigenous isolate TRC2 inoculated plants had the lowest total N fixed and were not significantly different when compared with uninoculated soyabean at IA (Fig. 4). Variety TGx1908-1F had significantly ($P<0.05$) lower amount of total N fixed compared to the other variety at OI (Fig. 4). The relationship between the total N fixed and grain yield was relatively strong and positive in each of the study sites (Fig. 5). Inoculation and variety had no significant effect ($P<0.05$) on % NDFA at UITRF and OI (Fig. 6). However, the % NDFA was significantly higher in IDC8 and R25B+IRj2180A inoculated plants than other plants at IA (Fig. 6).

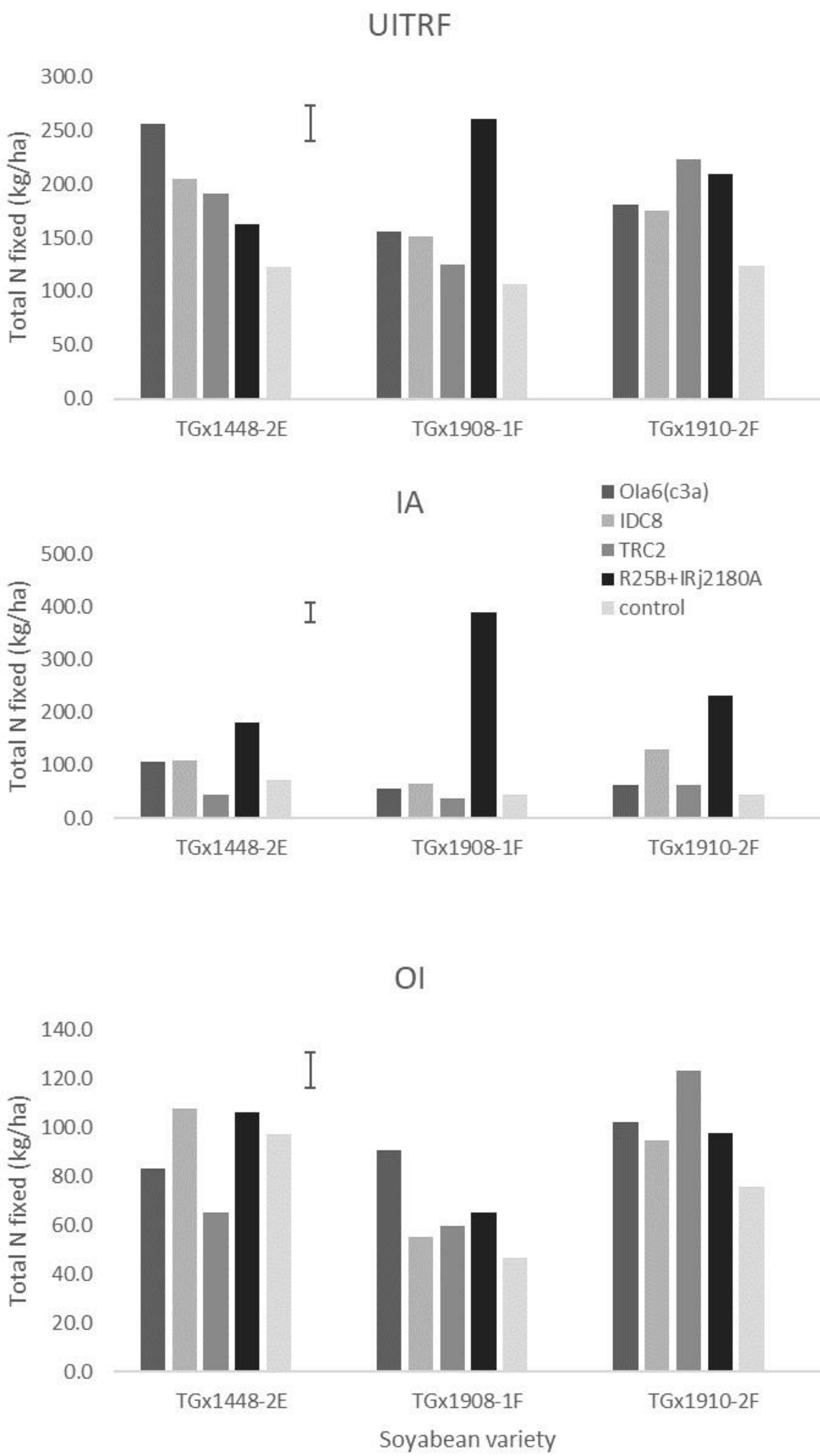
UITRF
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Total N fixed (kg/ha)
TGx1448-2E
TGx1908-1F
TGx1910-2F
IA
Ola6(c3a)
IDC8
TRC2
R25B+IRj2180A
control
500.0
400.0
300.0
200.0
100.0
0.0
Total N fixed (kg/ha)
TGx1448-2E
TGx1908-1F
TGx1910-2F
OI
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Total N fixed (kg/ha)
TGx1448-2E
TGx1908-1F
TGx1910-2F
Soyabean variety

Figure 4. Total N fixed by three soyabean varieties as a result of rhizobial inoculation in the three study sites. Bars represents SE of mean

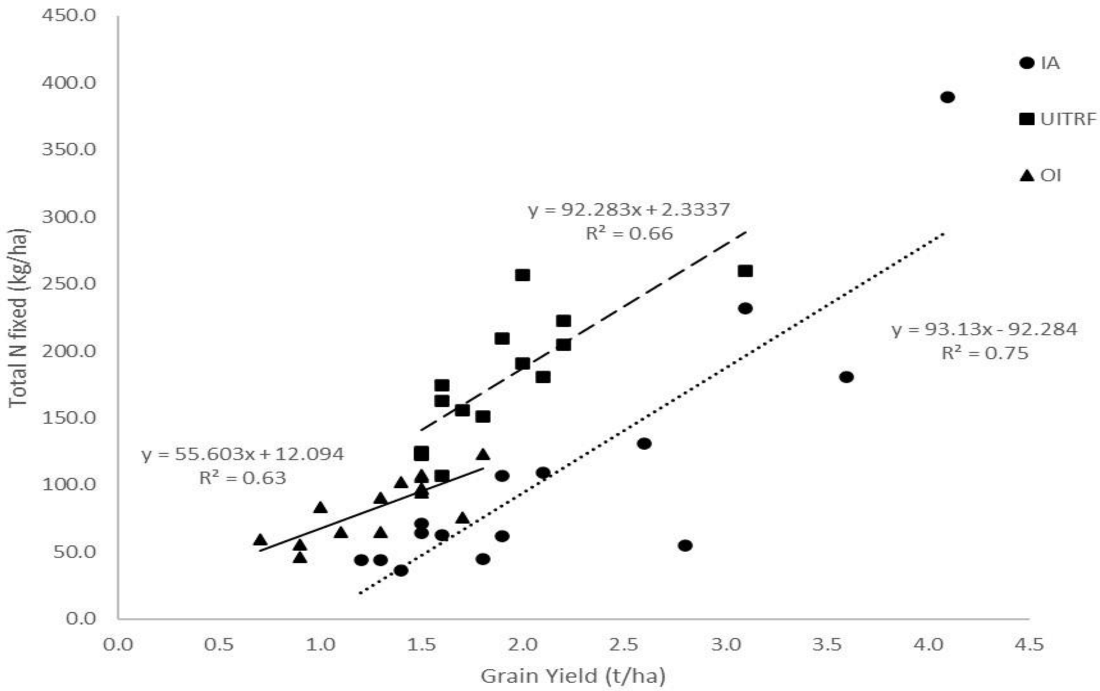

Figure 5. Relationship between grain yield and total N fixed in each of the study sites

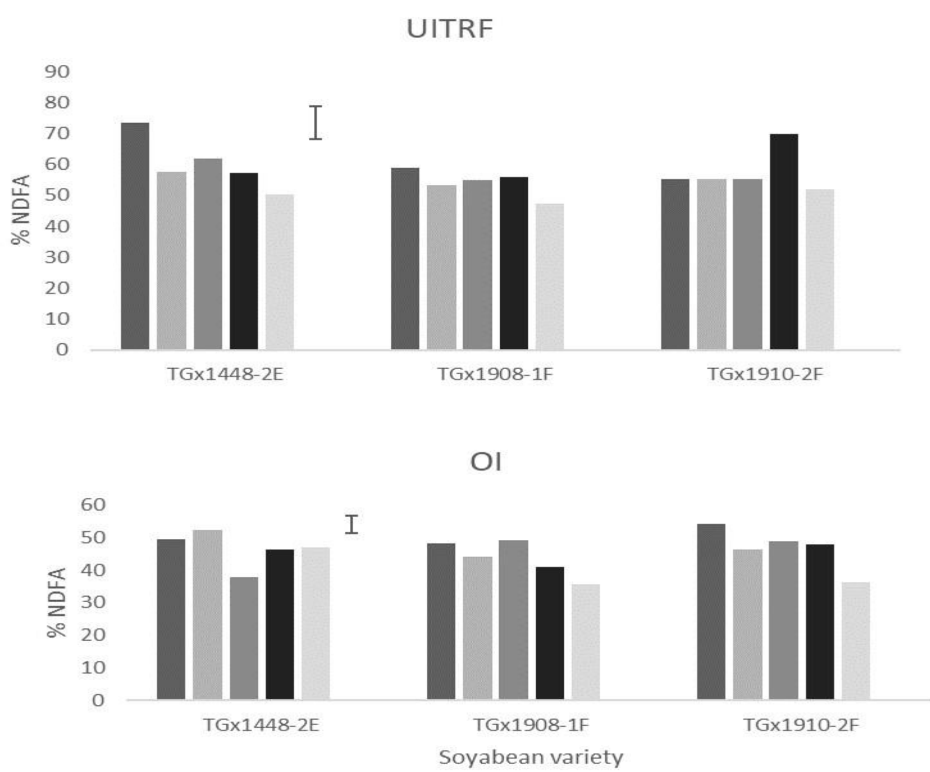

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Figure 6. The plant N derived from atmosphere (%NDFA) as affected by rhizobial inoculation of three soyabean varieties in three study sites. Bars represents SE of mean.

Discussion

Soyabean response to rhizobial inoculation is influenced by soil chemical and biological properties which includes pH, N and P availability and indigenous rhizobial population (Thies et al., 1992; Giller 2001; Osunde et al., 2003; Ronner et al., 2016). The rhizobial count of the three locations in our study was generally low with the highest of 13 cells per gram soil found at UITRF. Population of indigenous rhizobia in most tropical soils are low (Ahmad, 1981; Ahmad and Mchaughing, 1985, Sanginga, 2000a) and response of crops to inoculation is likely to occur when rhizobial count is less than 10 cells per gram of soil (Thies et al., 1991, 1992; Sanginga et al., 1996; Okogun and Sanginga, 2003). In our study, soyabean response to inoculation varied across locations. It was very clear in the pot experiment that rhizobial inoculation increased the number of nodules observed, both in sterile and non-sterile soil. The higher number of nodules observed in plants inoculated with exotic strains over other inoculated plants under non-sterile condition at UITRF and IA confirms the competitiveness of exotic strains against native rhizobia for nodule occupancy. Giller and Wilson (1991) reported that the most significant environmental factor limiting nodule occupancy of an introduced strain is the size of the indigenous rhizobial population in the soil, which was low in all the locations in this study.

The increased grain yield observed at IA in response to inoculation was probably due to its soil condition. High soil pH, total N and Ca and low rhizobial count are factors that likely complemented the effectiveness of inoculation in increasing soyabean yield in IA. Even with low available P in all the study sites, IA had highest content compared to the other study sites. The importance of adequate P for increased legume yield is well documented (Giller and Cadisch, 1995; Didagbé et al., 2014; Ronner et al., 2016). According to Vanlauwe et al. (2019), the overall mean effects of inoculation and P on grain yields of soybean when applied alone were often in the region of an extra 0.5t ha^{-1} of yield.

Higher soil pH also improves soil bacterial activities (Sanginga and Woomer, 2010) which can lead to higher soil organic matter, mineralization and nutrient cycling that are ingredients for improved plant growth and yield. Grain yield at UITRF was also improved by inoculation of TGx1448-2E and TGx1910-2F where rhizobial isolates TRC2, IDC8 and OISa-6e increased yield by more than 25% when compared to uninoculated plants. This increase was more than an average yield increase of 115kg ha^{-1} or 9.5% increase relative to the uninoculated treatments that was reported by van Heerwaarden et al. (2018).

Compatibility of strains with legume varieties and genotypes is an important factor that can affect effectiveness of legume symbioses (Bulland et al., 2005). It is important to note the effectiveness of inoculation of R25B+IRj2180A on TGx1908-1F which produced the highest grain yields in IA and UITRF. This exotic strain had outstanding adaptation across the locations with their influence on nodulation, total N fixed and uptake and grain yield. Vanlauwe et al. (2019) reported that most exotic strains used in inoculants have broad adaptability with outstanding performance in broad range of soil. The outstanding performance of R25B+IRj2180A may also be due to the superiority of combination of strains R25B and IRj2180A, an inoculant factor that has been reported to increase yield in legumes (Chibeba et al., 2018; Campo et al., 2009). In terms of compatibility, the indigenous isolate OIa6(c3a) and IDC8 with variety TGx1448-2E produced promising responses on grain yield and amount of N fixed and it would be a good idea to test the performance of the two strains combined on several varieties of soybean. The inoculation of the two isolates showed responses that were comparable to that of the exotic strains R25B+IRj2180A especially at UITRF. In fact, OIa6(c3a) and IDC8 inoculated TGx1448-2E fixed over 40 kg/ha N more than the exotic strains at UITRF. The efficacy of the inoculation of these two indigenous strains with TGx1448-2E in terms of N fixation and grain yield gives an insight into the potential use of indigenous isolates as elite strains to inoculate soybean for enhanced N fixation and

yield improvement. It is imperative to screen the rhizobial population for compatibility and effectiveness in the selection process of locally adapted strains for inoculant production for legumes.
There are reports on the compatibility and effectiveness of some indigenous strains for the improvement of biological N fixation in promiscuous soybean cultivars in sub Saharan Africa (Abaidoo et al., 2000, 2007; Klogo et al., 2015; Gyogluu et al., 2016). Legumes including soyabean often depend on soil N at the expense of N fixation when the soil N is at high or moderate level (Herridge and Rose, 2000; Beyan et al., 2018). The average % NDFA was generally low at IA (<35%), a location with moderate level of soil N, compared to the other two locations (OI, 45% and UITRF, 57%) with low level of soil N. Bender et al. (2015) also mentioned that the soil contributes a large portion of N accumulated in soybean despite their to fix N and this probably explains higher biomass N uptake (grain and shoot) at IA. The inoculation of all the soyabean varieties with R25B+IRj2180A and TGx1448-2E with OIa6(c3a) and IDC8 improved N uptake at IA. The total N accumulations in the grain and shoot of the three soybean varieties in the three locations were within the range reported by Flannery (1986). The high harvest index of N in the three locations corroborated previous studies on soybean (Bender et 2015; Barth et al 2018). This high accumulation in the grain represent a large removal of N from the soil and therefore inoculation of seed with effective inoculants is important for the sustainability of soybean production even with fertilizer input.

Conclusion

The indigenous rhizobial isolates OIa6(c3a), IDC8 and TRC2 had potentials for use as inoculant to increase soyabean production in the rainforest of Nigeria. Their ability to compete with the resident rhizobial population for nodule occupancy and improve grain yield and N fixation of some of the soyabean varieties even at low available P. Co-inoculation of R25B and IRj2180A in field trial was efficient at increasing nodulation and grain yield of the three soybean varieties. Critical to the efficiency of rhizobial inoculation in this study was soil biological and chemical properties and varietal differences of soyabean. Further studies on the performance of coinoculation of the indigenous isolates in soil with low and high rhizobial count is suggested.

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References

Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Borthakur, D., 2000. Bradyrhizobium spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. Int. J. Syst. Evol. Microbiol. 50, 225–234.

Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Dashiell, K.E., 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. http://dx.doi.org/10.1016/j.apsoil.2006.05.006. (ISSN: 09291393)

Ahmad, M. H., Mclaughing, W., 1985. Ecology and genetics of tropical Rhizobium species. Biotechnology Advance. 3: 155 – 170.

Ahmad, N., 1981. Growth of Leucaena leucocephala in relation to soil pH, nutrient levels and rhizobium concentration. Leucaena Research Report 2: 5 – 10.

Barth, G., Francisco, E., Suyama, J. T., Garcia, F., 2018. Nutrient Uptake Illustrated for Modern, High-Yielding Soybean. Better Crops/Vol. 102: 11-14

Bender, R.R., Haegele, J.W., Below F.E., 2015. Nutrient uptake, partitioning, and remobilization in modern soybean varieties. Agron. J. 107:563–573. doi:10.2134/agronj14.0435

Beyan, S.M., Wolde-meskel, E., Dakora, F.D., 2018. An assessment of plant growth and N2 fixation in soybean genotypes grown in uninoculated soils collected from different locations in Ethiopia. Symbiosis 75: 189203. https://doi.org/10.1007/s13199-018-0540-9

Bray, R. H., Kurtz, L. T., 1945. Determination of total organic and available form of phosphorus in soils. Soil Sci. J. 59: 39-45.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen -total. In: Page AL, Miller, RH, Keeney DR (eds), Methods of soil analysis, part 2. Amer. Soc. Agron. 9:595–624.

Bulland, A.O., Roughley, R. J. and Pulsford, D. J. 2005. The legume inoculant industry and inoculant quality control in Australia: 1953 – 2003. Aust. J. Exp. Agric. 45 (2-3): 127 – 140.

Campo, R.J., Araujo, R.S., Hungria, M., 2009. Nitrogen fixation with the soybean crop in Brazil: compatibility between seed treatment with fungicides and bradyrhizobial inoculants. Symbiosis 48, 154–163. http://dx.doi.org/10.1007/BF03179994. (ISSN: 1878-7665).

Chibeba, A.M., Kyei-Boahen, S., Guimarães, M.F., Nogueira, M.A., Hungria, M., 2018. Feasibility of transference of inoculation-related technologies: a case study of evaluation of soybean rhizobial strains under the agroclimatic conditions of Brazil and Mozambique. Agric. Ecosyst. Environ. 261, 230–240. https://doi.org/10.1016/j.agee.2019.106583

Didagbé, O.Y., Houngnandan, P., Sina, H., Zoundji, C.C., Kouelo, F.A., Lakou, J., Toukourou, F., Moussa, L.B., 2014. Response of groundnut (Arachis hypogaea L.) to exogenous Bradyrhizobium sp strains inoculation and phosphorus supply in two agroecological zones of Benin, West Africa. J. Exp. Biol. Agric. Sci. 2, 623–633.

Giller, K.E. and Wilson, K.J. 1991. Nitrogen fixation in Tropical cropping system CAB International, Wallingford, United Kingdom 313 Pp.

Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems, 2nd ed. CAB International, Wallingford.

Giller, K.E., Cadisch, G., 1995. Future benefits from biological nitrogen fixation: an ecological approach to agriculture. Plant Soil 174, 255–277. http://dx.doi.org/10. 1007/BF00032251.

Giller, K.E., Murwira, M.S., Dhliwayo, D.K., Mafongoya, P.L., Mpepereki, S., 2011. Soyabeans and sustainable agriculture in southern Africa. Int. J. Agric. Sustain. 9, 50–58. http://dx.doi.org/10.3763/ijas.2010.0548. (ISSN: 1747-762X).

Gyogluu, C., Boahen, S.K., Dakora, F.D., 2016. Response of promiscuous-nodulating soybean (Glycine max L. Merr.) genotypes to Bradyrhizobium inoculation at three field sites in Mozambique. Symbiosis 66 ISSN: 0334-5114 DOI: 10.1007/s13199-0150376-5.

Hardarson, G., Danso, S. K. A., 1993. Methods of measuring biological nitrogen fixation. Plant Soil 152, 19 – 23.

Herridge, D., Rose, I., 2000. Breeding for enhanced nitrogen fixation in crop legumes. Field Crop Res 65(2):229– 248. https://doi.org/10. 1016/S0378-4290(99)00089-1

Herridge, D. F., 1982. Legume Nitrogen fixation. In Methods to measure legume nitrogen fixaton. Workshop Handbook 1994.NWFP. Agriculture University Peshawar, Pakistan.Pp 38.

Herridge, D. F., Fraser, J.B., Peoples, M.B., 1990. Measurement of nitrogen fixation by soybean in the field using the ureide and natural ^{15}N abundance methods. Plant Physiol. 93, 708-716.

Hungria, M., Campo, R.J., Mendes, I.C., Graham, P.H., 2006. Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics: the success of soybean (Glycine max L. Merr.) in South America. In: Singh, R.P., Shankar, N., Jaiwal, P.K. (Eds.), Nitrogen Nutrition and Sustainable Plant Productivity. Studium Press, LLC Houston, pp. 43–93.

Hungria, M., Mendes, I.C., 2015. Nitrogen fixation with soybean: the perfect symbiosis? In: de Bruijn, F.J. (Ed.), Biological Nitrogen Fixation. John Wiley&Sons, Inc, New Jersey, pp. 1005–1019. http://dx.doi.org/10.1002/9781119053095. (ISBN: 978-1118-63704-3).

Klogo, P., Ofori, J.K., Amaglo, H., 2015. Soybean (Glycine max (L) Merill) promiscuity reaction to indigenous bradyrhizobia inoculation in some Ghanaian soils. Int. J. Sci. Tech. Res. 4, 306–313. http://dx.doi.org/10.1016/j.apsoil.2005.06.008. ISSN: 09291393. Nelson, D. W., Sommers, L. E., 1982. Total Carbon, Organic matter. In: methods of soil analysis part 2 Agronomy monograph 9 second Edition. A. L. Page. Ed. American society of Agronomy and Soil Science Society of Africa Madison, Wisconsin, pp. 539 – 579.

Ojo, A, Dare, M.O., Fagbola, O., Babalola, O., 2015. Variations in infectivity of indigenous rhizobial isolates of some soils in the rainforest zone of Nigeria, Arch. Agron. Soil Sci. 61:3, 371-380. DOI: 10.1080/03650340.2014.933811

Okalebo, J. R., Gathua, K. W., Woomer, P. L., 1993. Laboratory methods of soil and plant analysis: A working manual. Tropical Soil Biology and Fertility Programme.

Okereke, G. U., Onochie, C. C., 1996. Short communication. Screening of native and foreign Bradyrhizobium japonicum strains for high fixation in soybean. World J. Microbiol. Biotech. 12 (6), 639 – 641.

Okogun, J. A., Sanginga, N., 2003. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agro ecological zone of Nigeria? Biol. Fertil. Soils 38, 2631.

Osunde, A., Gwam, S., Bala, A., Sanginga, N., Okogun, J., 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. http://dx.doi.org/10.1007/ s00374-003-0611-8. (ISSN: 0178-2762).

Ranga-Rao, V., Ayanaba, A., Eaglesham, R. J. A., Thottappilly, G., 1984. Effects of Rhizobium Inoculation of field grown soybeans in Western Nigeria and assessment of inoculum persistence during a two-year fallow. Trop. Agric. 62 (2), 125 – 130.

Ronner, E., Franke, A.C., Vanlauwe, B., Dianda, M., Edeh, E., Ukem, B., Giller, K.E., 2016. Understanding variability in soybean yield and response to P-fertilizer and rhizobium inoculants on farmers' fields in northern Nigeria. Field Crop Res. 186, 133–145. https://doi.org/10.1016/j.fcr.2015.10.023.

Sanginga, N, Woomer, P.L., 2010. Integrated soil fertility management in Africa: principles, practices and developmental process (p. 13). Tropical soil biology and fertility Institute of the International Centre for tropical agriculture (TSBF-CIAT), Nairobi, pp 263

Sanginga, N., Abaidoo, R., Dashiell, K., Carsky, R.J., Okogun, A., 1996. Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. Appl. Soil Ecol. 3, 215–224. http://dx.doi.org/10.1016/0929-1393(95) 00089-5.

Sanginga, N., Lyasse, O., Singh, B. B., 2000. Phosphorus use, efficiency and nitrogen balance of cowpea breeding lines in a low P soil of derived savanna zone in West Africa. Plant Soil 220, 119 – 128.

Sanginga, N., Thottappilly, G., Dashiell, K., 2000. Effectiveness of rhizobia nodulating recent promiscuous soyabean selection in the moist savanna of Nigeria. Soil Biol. Biochem. 32, 127 – 133.

SAS. 2003. SAS/STAT .Guide for personal computers, Version 9.1 Edition,1028 pp Cary, NC. SAS. Institute Inc.

Smyth, A. J., Montgometry, R. F., 1962. Soils and land use in Central Western Nigeria. Ibadan Nigeria. Govt. of western Nigeria (Pub.) Ibadan 1962.

Somasegaran, P., Hohen, H. J., 1994. Handbook for Rhizobia: Method in Legume-Rhizobium Technology. University of Hawaii NifTAL Project 1000 Holomua Road Paia, Hi96779 – 9744 USA, 450 pp.

Thies, J.E., Singleton, P.W., Bohlool, B.B., 1991. Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. Appl. Environ. Microbiol. 57, 29–37 (ISSN: 0099-2240).

Thies,J.E., Bohlool, B.B., Singleton, P.W., 1992.Environmental effects on competition for nodule occupancy between introduced and indigenous rhizobia and among introduced strains. Can. J. Microbiol. 38, 493– 500. http://dx.doi.org/10.1139/m92081. (ISSN: 0008-4166).

USDA, 2006. A basic system of soil classification for making and interpreting soil survey Agriculture Handbook 436, Natural resources conservation service USD Awww3intersciencewiley.com/journal/18981858/abstract.

Van Heerwaarden, J., Baijukya, F., Kyei-Boahen, S., Adjei-Nsiah, S., Ebanyat, P., Kamai, N., Woldemeskel, E., Janampiu, F., Vanlauwe, B., Giller, K., 2018. Soyabean response to rhizobium inoculation across subSaharan Africa: patterns of variation and the role of promiscuity. Agric. Ecosyst. Environ. 261, 211–218.

Vanlauwe B., Hungria M., Kanampiua F., Giller K.E. (2019). The role of legumes in the sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for the future. Agric. Ecosyst. Environ. 284, (2019) 106583 https://doi.org/10.1016/j.agee.2019.106583