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Response of Groundnut and Soybean to Selected Elite Strains of Rhizobia on Farmers Fields Around Minna, Southern Guinea Savanna Zone of Nigeria

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Abstract: The increased use of symbiotic fixation of nitrogen by rhizobia should help in achieving increased yield of grain legume crops in a most economical way. A trial was conducted during the 2018 cropping season on four farmers' fields located in villages around Minna, using the SAMNUT 24 groundnut and TGX1835-10E soybean cultivars. The objective of the study was to observe the response of these legumes to some elite rhizobia strains that were evaluated in comparison with known reference inoculants and the indigenous soil rhizobia of the locality. The Treatments for soybean include: uninnoculated (control), elite strains NAK 128, NAK 84, RACA 6, IRJ 2180 A, RANI 22, USDA 532 C (Ref), USDA 110 (Ref) and 60 kg N ha^{-1}. While those for groundnut were; MJR 493, SBG 234, SSN 336, SNN 343, IGB 469, NC 92 (Ref) and 60 kg N ha^{-1}. All plots received a basal dose of 30 kg P ha^{-1} at planting, while the nitrogen fertilizer treatment was spot-applied as Urea in 2 splits (20 kg N ha^{-1} at 2 weeks after planting (WAP), and 40 kg N ha^{-1} at 4 WAP). Each treatment plot was 36 m^2 of six manually made ridges each of 6 m long with an inter-ridge spacing of 80 cm. The trial was laid out in a Randomized Complete Block Design (RCBD) and replicated three times on each farmer's field. Data were collected on plant height at 4, 6 and 8 WAP, Nodule number, nodule dry weight, shoot dry weight at mid-flowering, pod yield, grain yield and 100 grain weight at harvest. Maize was planted as reference crop to estimate the amount of N fixed by the legumes using the N-difference method. The MPN results showed a high population of native rhizobia in the soils. Groundnut plants inoculated with IGB 469 produced significantly higher pod and grain yield by about 30% respectively over the uninnoculated plants in Gidan Kwano. In the soybean experiment, it was observed that plants inoculated with RANI 22 produced significantly 60% and 56% higher nodule biomass than the uninnoculated plants in Gidan Kwano and Gurusu farm sites respectively. The results of this study indicated that response to inoculation by the legumes varied from one farm field to another and some of the elite strains stimulated increase in nodulation, shoot biomass, pod yield, grain yield and amount of N fixed which were significantly higher than the control.

Keywords: Rhizobial, Inoculation, Elite Strain, Nodulation, Farmers' Fields, N Uptake, N Fixation

1. Introduction

In spite of the great potentials of groundnut and soybeans to reduce food insecurity and reduce malnutrition in Nigeria, yields are generally lower than one ton per hectare. In Africa, South Africa is the highest producer of soybean with Nigeria being the second largest producer with a production of about 640,000 tons per year and this trend has been the same over the past five seasons with no increase and it is just above the annual consumption of 610,000 tons [6]. The projected soybean production of 346.6 million metric tons in 2017/2018 market year considered Brazil, Argentina and USA as the highest producers (38) and Nigeria was grouped under others. However, the global groundnut production in 2018 placed Nigeria and USA as the third largest producer after China and India and of these countries only Nigeria consumes most of the groundnut produced locally while others are primarily exporters (24). There is therefore a wide gap between what is currently being produced and what is needed due to fertility constraints especially nitrogen [39].

Since 2013 and 2014 cropping seasons, production of legumes in Nigeria has remained constant [16] and the population is increasing. Improving nitrogen fixation in grain legumes is central to improving agricultural production in Nigeria and to improve groundnut and soybean production, improved seeds have been produced, however that alone could not effectively increase yield to optimal potential of these legumes. Inoculation with elite rhizobia strains is being promoted to increase legume grain yield in tropical smallholder farmers. Combination of improved seeds of groundnut and soybean with these elite strains in Minna may increase the yield potential of these legumes. Studies carried out in Kabete, West of Nairobi, Kenya showed that an elite rhizobia strain (NAK 128) emerged the most promising producing about 1.5 t ha^{-1} of soybean and outperforming both the control and reference strain USDA 110 [41]. Also, some studies in Nigeria have shown responses of some promiscuous soybeans cultivars to inoculation by introduced rhizobia strain [32, 35]. Other studies focused on groundnut show sporadic responses due to their long adaptation and compatibility with indigenous rhizobia that naturally nodulates with it [7]. However, with mixed results obtained from previous inoculation studies in Minna [39] there is need to conduct trials with these elite strains of groundnut and soybean in Minna to identify the strains that would produce expected grain yields of one ton per hectare.

For the isolated bacteria to be considered as elite strain, it must have been screened by outperforming a known reference strain, optimal N level (60 - 80 kg N ha^{-1}) and compete favorably with indigenous rhizobia in pot experiments and under different farming conditions over a wide geographical range [41]. In this way, a wide diversity of rhizobia isolates will ensure a sustainable source of strains for commercial application into the future [27]. This was the main focus of the N2 Africa project sponsored by the Bill Gates and Melinda Gates Foundation that ended in June 2019. The aim of this study was to assess the response of groundnut and soybean to some selected elite strains of rhizobia on farmers' fields around Minna and to quantify the atmospheric nitrogen fixed by these rhizobia strains in symbiosis with groundnut and soybean.

2. Materials and Methods

Experimental Sites

The trial was carried out during the 2018 cropping season (June - September) on farmer's fields in four villages around Minna in the Southern Guinea Savanna Zone of Nigeria, (Latitude 9^0 41" N and Longitude 6^0 30" E). The Climate around Minna is sub humid tropical, with mean annual rainfall of 1284 mm (90% of the rainfall is between the month of June and August). The mean maximum temperature remains high throughout (33.5°C) particularly in February to March (28; 1) and an annually distinct dry season of about five months occurs from November to March. The physical features around Minna consist of granites, migmatites, gneisis and schists. Inselbergs of "Older Granites" and low hills of schists rise conspicuously above the plains and bedrock is deeply weathered and constitutes the major soil parent material [28]. The soil around Minna is mostly classified at the Order level as Alfisols [23].

Groundnut cultivation was carried out at Gidan Kwano (Lat.$9^0$31'589" N and Long. $6^0$26' 284"E), Kodoko (Lat.$9^0$34'855" N and Long. $6^0$32' 422"E) and Tutungo (Lat.$9^0$30'385" N and Long. $6^0$35' 474"E). Soybean was cultivated at Gidan Kwano and Kodoko with the same coordinates as for groundnut and Gurusu (Lat.$9^0$37'917" N and Long. $6^0$38' 877"E). These sites were chosen due to the low yield of legumes obtained from previous IITA studies in the area as a result of the low fertility status of the area.

Soil Sampling and Laboratory Studies

Prior to the commencement of the experiment, soil samples were collected with a soil auger (0-20 cm) systematically in a "W" shape pattern at 18 auger points from each experimental site and the auger was cleaned at the end of sample collection from each site with running tap water to reduce contamination. The samples were collected into sampling bags, thoroughly mixed together and bulked to form a composite. The collected samples were split into two, one kept in the refrigerator for microbial study and the second soil sample was air-dried, gently crushed and packed in jute sampling bags with appropriate labels and both (refrigerated and air-dried) samples were sent to International Institute of Tropical Agriculture (IITA), Ibadan for routine soil analysis.

The samples sent to IITA, Ibadan for routine soil analyses were analyzed using these methods: The hydrometer method [3] was used for the determination of particle size analysis. Soil pH was measured using a pH meter in the supernatant suspension of 1:2.5 soil and water mixture. Walkley and Black method [40] was used for soil organic carbon. The Kjeldahl method was used to measure total nitrogen in soil samples. Bray P1 method [10] was used to determine available phosphorus. Effective Cation Exchange Capacity was done by the summation method [16].

Land Preparation

At each farm site, land was cleared and ridges were made manually with local hoes. There was no use of chemicals of any kind prior to planting. After clearing, 6 m x 6 m plots were laid out using measuring tape, garden line and pegs.

Source of Experimental Materials

Seeds and fertilizers used for this trial; Maize, Soybean cultivar (TGx 1835-10E), Groundnut cultivar (SAMNUT 24), Urea, Single Super Phosphate (SSP) and peat-based rhizobia inoculants were obtained from IITA Ibadan.

Treatments and Experimental Design

The Treatments (Isolates / Reference inoculants) used for soybean (TGx 1835-10E) were; NAK 128, NAK 84, RACA 6, IRJ 2180 A, RANI 22, USDA 532 C (Ref) and USDA 110 (Ref). While Treatments (Isolates / Reference inoculants) used for groundnut (SAMNUT 24) were; MJR 493, SBG 234, SSN 336, SNN 343, IGB 469 and NC 92 (Ref), with an absolute control or negative N (uninnoculated) and a standard control or positive N (60 kg N ha^{-1}) supplied as urea for the N treatment, 20 kg N ha^{-1} and 40 kg N ha^{-1} were applied to the plots at 2 and 4 weeks after planting (WAP) respectively. A basal application of 30 kg P ha^{-1} was applied as single super phosphate (18% P_2O_5) to the maize, groundnut and soybean plots at planting.

The experiment was a single factor experiment fitted in a Randomized Complete Block Design (RCBD) and replicated three times on each of the farmers' field. The sizes of the plots were 36 m^2 (6 m x 6 m) consisting of 6 manually made ridges with an inter-ridge spacing of 80 cm at a planting distance of 5 cm and spacing between plots and blocks was 1 m and 2 m, respectively. The groundnut and soybean had a total of 24 (1 x 8 x 3) and 27 (1 x 9 x 3) plots on each field respectively.

Seed Inoculation and Agronomic Practices

The groundnut and soybean seeds were moistened with distilled water and mixed with each of the inoculants in a plastic bowl at the rate of 5 g of the inoculants per one kilogram (1 kg) of the seeds just before planting. The content in the bowl was stirred gently until the seeds were evenly coated with inoculant. Planting of the seeds were done immediately after coating with the inoculants, to avoid direct exposure of the coated seeds to sunlight. Four seeds of the legumes were planted per stand at an inter-row spacing of 5 cm. The plants were later thinned to two per stand at 2 WAP. The un-inoculated seeds and those receiving Nitrogen were planted first to avoid contamination. Maize was planted beside each plot to serve as reference crop for the quantification of atmospheric nitrogen fixed using the N difference method. The maize seeds were sown on a single ridge at the rate of 4 seeds per stand (50 cm apart) and later thinned to 2 per stand at 2 WAP. Weeding was done (with hoe) as at when necessary throughout the growth stage of the crops to reduce competition between the plants and weed.

Shoot dry weight, nodule number and dry weight of the legume plants

Destructive sampling was done at 50% flowering; the plants in the four inner ridges which served as the net plot were used for assessment at each sampling site. Eighth plants were randomly selected from the net plots and cut at soil level and roots were gently dug out with hand trowel. Thereafter, the plant roots were washed through a fine sieve with water to remove soil particles and organic debris, packed together and taken to the laboratory for oven drying. Nodules were detached from the roots of the eight selected plants, counted and mean number per plant was calculated by dividing the total number of nodules by 8. The fresh nodules and packed shoots were then oven drying at 70°C for 48 hours and the nodules and shoot dry weights were recorded.

Shoot dry weight of maize plant at 50% tasseling

Similarly, at 50% tasseling stage, the shoot of two maize plants beside each of the legume plot were cut at the ground level using scissors and oven dried at 70°C for 48 hours and their dry weight recorded.

Determination of amount of N fixed

To assess Biological Nitrogen Fixation in this study, maize fields was established as reference crop. The determination of N Fixed was carried out using Total Nitrogen Difference (TND) method by comparing total nitrogen of the legume with that of the reference plant [42].

$$\text{Total Nitrogen yield in plant} = \frac{\text{shoot dry matter weight (g) x \% Nitrogen in plant}}{100}$$

Amount of Nitrogen Fixed = Total N yield in legume – Total N yield in reference crop

Determination of yield and yield components at harvest

At all locations, a net plot size of 8 m^2 carved out in the middle of the two inner most ridges served as the harvest area. The legumes were harvested at full physiological maturity when the leaves of legumes were yellowish and the pods of soybean where golden yellow at 12 WAP. After harvest, the pods were weighed to obtain pod yield, threshed, winnowed and weighed on an electronic weighing balance to obtain grain yield per 8 m^2 and later extrapolated to per hectare. The grains were oven-dried at 70 °C for 48 hours and hundred seeds from each treatment were randomly selected and weighed. This was repeated three times and the mean 100-grain weight determined.

Data Analysis

Data collected was subjected to analysis of variance (ANOVA) using the SAS statistical package program version 9.4m^6 [34]. Duncan Multiple Range Test was used to separate treatment means ($P < 0.05$).

3. Results and Discussion

Soil physical, chemical and biological characteristics of the farmers' fields

The results of the physical, chemical and biological properties of the soils are presented in Table 1. The farm sites were generally sandy loam in texture. The soil reaction was slightly acid (6.1 in Tutungo to 6.38 in Gurusu and Gidan Kwano), and low total N in the four experimental sites (less than 0.1). The available P ranges from 2.00 mg kg^{-1} in Kodoko to 4.66 mg kg^{-1} in Tutungo. The Soil Organic Carbon (SOC) values were rated low in all the experimental sites (0.15 g kg^{-1} in Tutungo to 2.31 g kg^{-1} in Gurusu). Calcium dominated the exchange complexes and it was medium in all the fields except in Gurusu where it was rated low (1.62 $cmolkg^{-1}$). Magnesium was high in Gurusu and Tutungo but was rated medium in the other fields while Potassium was generally low and Sodium was high in the four sites. The Effective Cation Exchange Capacity (ECEC) of the fields ranges from 3.9 $cmolkg^{-1}$ in Gurusu to 5.04 $cmolkg^{-1}$ in Tutungo and it was rated low as they were less than 6.0 $cmolkg^{-1}$. The Most Probable Number (MPN) results obtained in these experimental sites showed a high population of native rhizobia in the soils ranging from 1.1 x 10^5 cells g^{-1} of soil in Gurusu to 4.3 x 10^5 cells g^{-1} of soil in Gidan Kwano.

Nodule number and nodule dry weight of groundnut

Results of nodule number and nodule dry weight are shown in Table 2. In Gidan Kwano and Kodoko there were significant ($p<0.05$) difference in nodule number as the plants inoculated with SNN 343 had the most nodule number (139 nodules) in Gidan Kwano which was significantly higher than the nodule number produced by plants inoculated with NC 92 but was similar to the others. This trend was however similar to what was observed in Kodoko where the plants inoculated with SNN 343 had the most nodule number (94 nodules) and it was significantly higher than the nodule numbers observed in the plants inoculated with NC 92 and the plants that received 60 kg N ha^{-1} but was similar to the others. In Tutungo, there was no significant difference in nodule number and nodule dry weight and this was similar to nodule dry weight in Gidan Kwano site. However, in Kodoko there was significant difference among the treatment plots and plants inoculated with SNN 343 and IGB 469 were significantly higher than plants that received 60 kg N ha^{-1} but was similar to the others.

Shoot dry weight and amount of N fixed by groundnut

The results of groundnut dry shoot weight and amount of N fixed are presented on Table 3. The shoot dry weight of groundnut was not significantly ($p>0.05$) different in all the treated plants in Gidan Kwano and Tutungo. However, plants inoculated with SNN 343 had the highest shoot dry weight (7.2 g) in Gidan Kwano while plants that received 60 kg N ha^{-1} had the highest (5.6 g) in Tutungo. In Kodoko, significant differences were observed and plants inoculated with SNN 36 and the uninnoculated plants had the lowest shoot dry weight of 3.5 g and 3.6 g respectively which were significantly lower than the others.

Table 1. *Soil physical, chemical and biological properties of the four farmers' fields.*

Farmers' Fields				
Soil parameters	**Gidan-kwano**	**Kodoko**	**Gurusu**	**Tutungo**
Particle size distribution (g kg^{-1})				
Sand	660	710	760	670
Silt	185	160	110	205
Clay	155	130	130	125
Textural Class	SL	SL	SL	SL
pH (H_2O) 1:2.5	6.38	6.20	6.38	6.10
SOC (g kg^{-1})	1.32	1.32	2.31	0.15
N (g kg^{-1})	0.009	0.009	0.02	0.034
Available P (mg kg^{-1})	3.15	2.00	2.26	4.66
Exchangeable bases (cmolkg^{-1})				
Ca	2.78	2.20	1.62	2.25
Mg	0.65	0.92	1.16	1.72
K	0.11	0.13	0.07	0.11
Na	0.79	0.82	0.85	0.86
Exch. Acidity (cmolkg^{-1})	0.20	0.30	0.20	0.10
ECEC (cmolkg^{-1})	4.53	4.37	3.90	5.04
Rhizobia population (cell g^{-1})	4.3x10^5	1.1x10^8	1.1x10^5	2.3x10^5

Sl= Sandy loam, ECEC= Effective Cation Exchange Capacity

Table 2. *Nodule Number and Nodule Dry Weight of Groundnut Grown on Farmers' Fields in Gidan Kwano, Kodoko and Tutungo as affected by selected Elite Rhizobia Strains.*

Treatments	**GIDAN KWANO**		**KODOKO**		**TUTUNGO**	
	Nod No.	**Nod DW**	**Nod No.**	**Nod DW**	**Nod No.**	**Nod DW**
	No plant^{-1}	**(g plant^{-1})**	**No plant^{-1}**	**(g plant^{-1})**	**No plant^{-1}**	**(g plant^{-1})**
CONTROL	104^{ab}	0.1^{a}	73^{abc}	0.06^{ab}	71^{a}	0.07^{a}
60 kg N ha^{-1}	109^{ab}	0.1^{a}	51^{c}	0.03^{b}	91^{a}	0.05^{a}
NC92 (ref)	83^{b}	0.1^{a}	61^{bc}	0.07^{ab}	111^{a}	0.07^{a}
SNN 36	97^{ab}	0.1^{a}	83^{ab}	0.07^{ab}	100^{a}	0.06^{a}
MJR 493	108^{ab}	0.1^{a}	70^{abc}	0.07^{ab}	119^{a}	0.07^{a}
SNN343	139^{a}	0.1^{a}	94^{a}	0.09^{a}	113^{a}	0.07^{a}
SBG234	97^{ab}	.1^{a}	71^{abc}	0.06^{ab}	103^{a}	0.07^{a}
IGB469	101^{ab}	0.1^{a}	83^{ab}	0.09^{a}	105^{a}	0.8^{a}
SE±	66.3	0.0005	25	0.0005	79	0.0002

Means with the same letters on the same column are not significantly different at ($p<0.05$)

Table 3. *Shoot Dry Weight and Amount of N Fixed by Groundnut on Farmers' Fields in Gidan Kwano, Kodoko and Tutungo as affected by selected Elite Rhizobia Strains3.*

Treatments	GIDAN KWANO		KODOKO		TUTUNGO	
	Sht dwt	N fixed	Sht dwt	N fixed	Sht dwt	N fixed
	(t ha^{-1})	(kg ha^{-1})	(t ha^{-1})	(kg ha^{-1})	(t ha^{-1})	(kg ha^{-1})
CONTROL	6.20^{a}	20.80^{c}	3.60^{b}	14.16^{b}	5.3^{a}	3.45^{cd}
60kg Nha^{-1}	6.90^{a}	46.60^{a}	4.40^{ab}	33.64^{a}	5.6^{a}	10.61^{a}
NC 92 (ref)	5.40^{a}	23.10^{c}	3.90^{ab}	24.64^{a}	4.5^{a}	2.28^{cd}
SNN36	6.10^{a}	26.10^{bc}	3.50^{b}	30.13^{a}	5.4^{a}	5.67^{bc}
MJR493	6.00^{a}	29.80^{bc}	4.70^{ab}	25.45^{a}	5.3^{a}	5.67^{bc}
SNN343	7.20^{a}	49.50^{a}	4.10^{ab}	27.73^{a}	4.0^{a}	1.08^{d}
SBG234	6.80^{a}	34.00^{b}	4.10^{ab}	34.11^{a}	4.5^{a}	8.70^{ab}
IGB469	7.10^{a}	45.80^{a}	4.90^{a}	28.99^{a}	4.5^{a}	9.09^{ab}
SE±	1.80	3.75	0.44	3.56	0.9	1.23

Means with the same letters on the same column are not significantly different at ($p<0.05$)

The amount of N fixed in Kodoko had significant differences. The uninnoculated control plants significantly fixed lower N than the others. This was different in Tutungo as SNN 343 was the lowest and it was significantly lower than plants that received 60 kg N ha^{-1}, SBG 234 and IGB 469. It was also noted that plants that received 60 kg N ha $^{-1}$ fixed significantly two times more N and three times more N than the uninnoculated plants in Gidan Kwano and Tutungo respectively.

Pod yield, grain yield and 100 seed weight of groundnut

The results for the pod yield, grain yield and 100 seed weight of groundnut are shown in Table 4. In Kodoko and Tutungo there were no significant differences in the pod yield of groundnut irrespective of the treatment. However, in Gidan Kwano plant inoculated with IGB 469 had the highest pod yield (1.1t ha^{-1}) which differs significantly from the others except plants in the plots that were inoculated with SNN 36, and SBG 234. There was no significant ($p>0.05$) difference in grain yield in Kodoko and Tutungo. In Gidan Kwano, the lowest grain yield was in control plants and plants inoculated with NC 92 (0.41 tha^{-1}) and it was similar to the others except for the plants inoculated with IGB 469 (0.58 t ha^{-1}) which had the highest grain yield.

The 100 seed weight in Kodoko and Tutungo were similar to pod yield (no significant differences). In Gidan Kwano, there were significant differences in 100 seed weight and plants inoculated with SNN 36 and IGB 469 had the highest 100 seed weight (38.3 g) and was similar to the others except for plants that received 60 kg N ha^{-1}.

Table 4. *Pod yield, Grain yield and 100 seed weight of groundnut on farmers' fields in Gidan Kwano, Kodoko and Tutungo as affected by selected elite rhizobia strains.*

Treatments	GIDAN KWANO			KODOKO			TUTUNGO		
	Pod yield	grain yield	100seed wt	Pod yield	grain yield	100seed wt	Pod yield	grain yield	100seed wt
	(t ha^{-1})	(t ha^{-1})	(g)	(t ha^{-1})	(t ha^{-1})	(g)	(t ha^{-1})	(t ha^{-1})	(g)
CONTROL	0.77^{dc}	0.41^{b}	33.6^{ab}	0.74^{a}	0.36^{a}	38.1^{a}	0.87^{a}	0.38^{a}	37.9^{a}
60KgN ha^{-1}	0.79^{bcd}	0.43^{b}	31.3^{b}	0.74^{a}	0.32^{a}	36.4^{a}	0.88^{a}	0.39^{a}	35.9^{a}
NC 92 (ref)	0.69^{d}	0.41^{b}	33.5^{ab}	0.73^{a}	0.33^{a}	36.8^{a}	0.79^{a}	0.36^{a}	39.2^{a}
SNN36	0.97^{ab}	0.52^{ab}	38.3^{a}	0.67^{a}	0.31^{a}	36.6^{a}	0.93^{a}	0.35^{a}	36.7^{a}
MJR493	0.82^{bcd}	0.43^{b}	34.6^{ab}	0.71^{a}	0.31^{a}	37.6^{a}	0.94^{a}	0.37^{a}	39.0^{a}
SNN343	0.89^{bc}	0.48^{ab}	36.5^{a}	0.65^{a}	0.29^{a}	38.6^{a}	0.75^{a}	0.33^{a}	36.9^{a}
SBG234	0.95^{abc}	0.51^{ab}	34.5^{ab}	0.81^{a}	0.37^{a}	37.3^{a}	0.85^{a}	0.35^{a}	37.4^{a}
IGB469	1.1^{a}	0.58^{a}	38.3^{a}	0.78^{a}	0.35^{a}	37.7^{a}	0.83^{a}	0.33^{a}	31.2^{a}
SE±	0.009	0.004	6.65	0.006	0.002	2.7	00.023	0.004	9.0

Means with the same letters on the same column are not significantly different at ($p<0.05$)

Nodule number and nodule dry weight of soybean

Results of nodule number and nodule dry weight are shown in Table 5. In Gidan Kwano plants inoculated with IRJ 2180 A had the most nodule number (114) and was similar to nodule numbers in the plants inoculated with RANI 22 and USDA 110 but significantly higher than others. In Kodoko a similar trend was observed as the most nodule number was in plants inoculated with RACA 6 (121) and was similar to the nodule numbers in plants inoculated with USDA 532 C, RANI 22, IRJ 2180 A and USDA 110 but was significantly higher than the others. In Gurusu, RANI 22 had the most nodule number (86) which was significantly similar to the others except nodule number of plants that received 60 kg N ha^{-1} (48).

In Gidan Kwano, plants inoculated with RANI 22 had the highest nodule dry weight of 0.96 g and it was similar to nodule dry weight of plants inoculated with NAK 84, IRJ 2180 A and RACA 6 and differs significantly from the others and this similar trend was observed in Kodoko and Gurusu. In Kodoko, RACA 6 had the highest nodule dry weight (0.60 g) which was similar to nodule dry weight of plants inoculated with NAK 84, RANI 22 and IRJ 2180 A but significantly higher than the others while in Gurusu, RANI 22 had the highest nodule dry weight (0.64 g) and was similar to plants inoculated with NAK 84, USDA 532 C, IRJ 2180 A and RAC 6.

Table 5. *Nodule Number and Nodule Dry Weight of Soybean grown on Farmers' Fields in Gidan Kwano, Kodoko and Gurusu as affected by selected Elite Rhizobia Strains.*

Treatments	**GIDAN KWANO**		**KODOKO**		**GURUSU**	
	Nod No.	**Nod dwt**	**Nod No.**	**Nod dwt**	**Nod No.**	**Nod dwt**
	No plant^{-1}	**g plant^{-1}**	**No plant^{-1}**	**g plant^{-1}**	**No plant^{-1}**	**g plant^{-1}**
CONTROL	32^{c}	0.38^{cd}	21^{d}	0.13^{ed}	51^{ab}	0.28^{ed}
60 kg N ha^{-1}	26^{c}	0.23^{d}	15^{d}	0.08^{e}	48^{b}	0.24^{e}
NAK128	81^{b}	0.51^{cd}	78^{bc}	0.39^{cb}	73^{ab}	0.45^{bcd}
NAK84	83^{b}	0.84^{ab}	73^{c}	0.46^{abc}	64^{ab}	0.50^{abc}
USDA532C	79^{b}	0.48^{cd}	85^{abc}	0.30^{dc}	78^{ab}	0.47^{abc}
RANI 22	93^{ab}	0.96^{a}	116^{ab}	0.58^{ab}	86^{a}	0.64^{a}
IRJ2180A	114^{a}	0.82^{ab}	84^{abc}	0.40^{abc}	66^{ab}	0.53^{ab}
USDA110	92^{ab}	0.63^{bc}	80^{abc}	0.33^{cd}	63^{ab}	0.34^{ade}
RACA6	80^{b}	0.89^{ab}	121^{a}	0.60^{a}	68^{ab}	0.54^{ab}
SE±	223.67	0.03	493.85	0.01	317.70	0.008

Means with the same letters on the same column are not significantly different at ($p<0.05$)

The plants in Gurusu inoculated with RANI 22 was significantly different in shoot dry weight (10.1 g) from the plants inoculated with NAK 128, RACA 6 and the uninnoculated control plants but similar to the others. The amount of N fixed by soybean was significantly different ($P<0.05$) in Gidan Kwano and Kodoko fields. The plants inoculated with USDA110 fixed more N in Gidan Kwano and it was significantly higher than uninnoculated control, NAK128 and USDA532C. In Kodoko, NAK 128 and IRJ 21805A fixed the least amount of N and they were significantly lower than what was fixed by NAK 84 and RANI 22.

Pod yield, grain yield and 100 seed weight of soybean

The results of the pod yield, grain yield and 100 seed weight of soybean are shown in Table 7. In Gurusu there were no significant differences in the pod yield of soybean irrespective of the treatment. However, in Gidan Kwano plants that received 60 kg N ha^{-1} had the highest pod yield (18.4 t ha^{-1}) which was significantly higher than the uninnoculated plants but similar to the others. In Kodoko, RACA 6 had the highest pod yield (18.5 t ha^{-1}) which was significantly higher than pod yield of plants inoculated with IRJ 2180 A (12.0 t ha^{-1}). There was no significant ($p>0.05$) difference in grain yield in Gidan Kwano and Gurusu. In Kodoko, the highest grain yield was in plants inoculated with RACA 6 (1.1 t ha^{-1}) and it was similar to the others except for plants that received 60 kg N ha^{-1}. In Gidan Kwano, plants inoculated with USDA 110 had the highest 100 seed weight (25.4 g) that was similar to the others except plants that received 60 kg N ha^{-1}, while in Kodoko the 100 seed weight had no significant difference as was in the case with Gurusu.

Table 6. *Shoot Dry Weight and amount of N Fixed by Soybean grown on Farmers' Fields in Gidan Kwano, Kodoko and Gurusu as affected by selected Elite Rhizobia Strains.*

Treatments	**GIDAN KWANO**		**KODOKO**		**KODOKO**	
	Sht dwt	**N fixed**	**Sht dwt**	**N fixed**	**Sht dwt**	**N fixed**
	(t ha^{-1})	**(kg ha^{-1})**	**(t ha^{-1})**	**(kg ha^{-1})**	**(t ha^{-1})**	**(t ha^{-1})**
CONTROL	9.8^{ab}	23.8^{bc}	$6.7b^{c}$	6.2^{cd}	7.4^{c}	24.1^{a}
N 60 Kg	11.5^{a}	29.1^{ab}	9.1^{ab}	8.6^{bc}	9.6^{ab}	36.9^{a}
NAK128	7.3^{b}	22.8^{bc}	5.9^{c}	4.3^{d}	7.6^{c}	22.8^{a}
NAK84	8.4^{ab}	28.2^{ab}	7.5^{abc}	15.9^{a}	8.7^{abc}	24.9^{a}
USDA532C	6.6^{b}	17.1^{c}	6.1^{c}	9.5^{b}	9.9^{ab}	40.8^{a}
RANI 22	9.2^{ab}	29.8^{ab}	9.0^{ab}	16.0^{a}	10.1^{a}	33.6^{a}
IRJ2180A	8.3^{ab}	27.4^{abc}	5.3^{c}	3.8^{d}	8.3^{abc}	39.9^{a}
USDA110	9.7^{ab}	34.8^{a}	6.3^{c}	5.5^{cd}	8.8^{abc}	28.4^{a}
RACA6	7.6^{b}	28.3^{ab}	9.5^{a}	$7.0b^{cd}$	8.2^{bc}	26.3^{a}
SE±	3.39	3.63	2.13	1.15	0.93	6.48

Means with the same letters on the same column are not significantly different at ($p<0.05$)

Table 7. *Pod yield, Grain yield and 100 seed weight of Soybean grown on Farmers' Fields in Gidan Kwano, Kodoko and Gurusu as affected by selected Elite Rhizobia Strains.*

Treatments	GIDAN KWANO			KODOKO			GURUSU		
	Pod yield	**grain yield**	**100seed wt**	**Pod yield**	**grain yield**	**100seed wt**	**Pod yield**	**grain yield**	**100seed wt**
	(t ha^{-1})	**(t ha^{-1})**	**(g)**	**(t ha^{-1})**	**(t ha^{-1})**	**(g)**	**(t ha^{-1})**	**(t ha^{-1})**	**(g)**
CONTROL	12.7^{b}	0.8a	24.2^{ab}	13.9^{abc}	0.8^{ab}	24.4^{a}	16.3^{a}	1.1^{a}	25.4^{a}
60KgN ha^{-1}	18.4^{a}	1.1^{a}	22.1^{b}	13.5^{abc}	0.7^{b}	23..3^{a}	18.7^{a}	1.1^{a}	24.2^{a}
NAK 128	15.7^{ab}	1.0^{a}	22.9^{ab}	16.7^{abc}	0.9^{ab}	25.8^{a}	16.6^{a}	0.9^{a}	26.3^{a}
NAK 84	15.3^{ab}	0.9^{a}	38.3^{a}	16.9^{ab}	0.9^{ab}	23.1^{a}	17.0^{a}	1.0^{a}	25.4^{a}
USDA532C	14.4^{ab}	0.9^{a}	25.2^{ab}	15.6^{abc}	0.9^{ab}	22.3^{a}	20.9^{a}	1.2^{a}	28.3^{a}
RAN I22	15.6^{ab}	1.0^{a}	22.3^{ab}	16.8^{ab}	1.0^{a}	25.4^{a}	18.1^{a}	1.1^{a}	25.6^{a}
IRJ 2180 A	16.9^{ab}	1.0^{a}	24.8^{ab}	12.0^{c}	0.8^{ab}	25.1^{a}	18.3^{a}	1.0^{a}	26.4^{a}
USDA 110	14.5^{ab}	1.0^{a}	25.4^{a}	14.3^{abc}	0.9^{ab}	24.9^{a}	17.3^{a}	1.2^{a}	26.0^{a}
RACA 6	16.5^{ab}	1.0a	23.6^{ab}	18.5a	1.1^{a}	22.5^{a}	20.7a	1.0a	24.9a
SE±	5.83	0.03	2.69	5.99	0.03	4.85	6.40	0.04	7.70

Means with the same letters on the same column are not significantly different at ($p<0.05$)

4. Discussion

Soil physical, chemical and biological properties of the farmers' fields

The results of the initial soil properties of the study area shows the characteristics of savanna soils which are usually low in soil organic carbon and total N contents [2]. The soil texture in the study areas were sandy loam and are suitable for legume cultivation. The soil pH was slightly acid and suitable for most crops [9] suggesting that the sites were suitable for legumes cultivation. The Soil Organic Carbon (SOC) values were rated low in all the experimental sites (0.15 g kg^{-1} in Tutungo to 2.31 g kg^{-1} in Gurusu) which may be as a result of residue removal as practiced in the area. This was not in agreement with the work of Lawal *et al.* [23]. They observed higher SOC around Minna perhaps by improved management system that included return of plant residue. The values of N obtained in this study indicate low N in the study area [15]. Low level of N in rhizobia trial enhances symbiotic association between bacteria and legumes. Several researches have shown that low N will increase nodule number and nodule dry weight and 40 – 60 kg N ha-1 will suppress the ability of rhizobia to nodulates [4, 8, 11, 32]. However, this low N content of the soil may be as a result of residue removal as practiced by the local farmers in the study area. The available P content of the experimental sites was low [5, 15] and cultivation of legumes will need P fertilization [23]. The Effective Cation Exchange Capacity (ECEC) of the soil was generally very low in all the experimental sites and this means low fertility status of the soil [20].

The Most Probable Number (MPN) results obtained in these experimental sites showed a high population of native rhizobia in the soils ranging from 1.1 x 10^{5} cells g^{-1} of soil in Gurusu to 4.3 x 10^{8} cells g^{-1} of soil in Gidan Kwano which is similar to the result of previous study in soils around Minna [39]. Soils where native rhizobia are greater than 10^{3} cells g^{-1} of soil is adequate to serve as control and the native rhizobia can be very competitive and difficult to be outperformed by introduced rhizobia [36, 41] thus making the experimental site suitable for this trial.

Effect of Elite Rhizobia Strains on groundnut

The response of groundnut to inoculation by these elite rhizobia strains varied from one farm field to another and this observation is in agreement with previous rhizobia studies (18; 41). However, the result of groundnut nodulation obtained indicated that uninnoculated plants did not show any significant difference from inoculated plants irrespective of location. This shows that the indigenous strains were very competitive in terms of nodule formation. In Gidan Kwano, plants that received 60 kg N ha^{-1} had nodule numbers that were high (109) and this high nodule number from N treated plot is different from previous work with legumes [18, 33].

Plants inoculated with IGB 469 produced significantly higher pod and grain yield by 30% over the uninnoculated plants in Gidan Kwano and 27% heavier shoot biomass over the control in Kodoko. The NC 92 reference strain was selected because it has given outstanding performance over a wide range of geological locations and the isolates with similar yield values with it were considered as elite strains [18]. This was not the case when similar elite strain was used in this trial as the results varied from one field to another. In a similar groundnut trial, plants that received 20 kg N ha^{-1} gave higher values (in all the parameters recorded) than the NC 92, MJR 518, SBG 274 and uninnoculated control [33, 18] suggesting that location or environmental condition may affect the performance of the elite strains.

The result obtained indicated that the amount of N fixed differs significantly from one elite strain to another. For example, plants inoculated with SNN 343 and IGB 469 fixed more N than the control in Gidan Kwano. The amount of N fixed across the fields was in the range of 1.1 - 49.5 kg N ha^{-1} which was lower than the expected range of 38 - 62 kg N ha^{-1} that was obtained from previous work [6, 17]. The results obtained also show that plants that received 60 kg N ha^{-1} fixed more N than the uninnoculated plants in the trial sites. This result varies from the expected as reported by several others. The probability that the N content of the urea fertilizer used in this study may have been affected due to long storage and poor quality cannot be over looked. There have been reported cases of farmers in the area complaining of poor response of crops to fertilizers, especially Urea. Nodulation and N fixation by legumes is adversely affected by increased rates of nitrate. Thornton and Nicole [37] were one of the first observers of the effect of high concentration of nitrate on legumes. High concentration of nitrate affects nodulation and N fixation by inhibiting root hair formation, curling of root hairs and damaging of nodules which leads to early nodule senescence [12]. Inorganic N inhibits the *rhizobia* infection process and also inhibits N fixation [4, 43]. The inhibitory effects of mineral N on nodulation and N fixation of legumes are clear at high concentrations [19]. Olsson *et al.* [31] showed that N fixed by legumes was reduced when grown in high concentration of nitrogen.

Effect of Elite Rhizobia Strains on soybean

There was significant ($P > 0.05$) difference in nodulation, shoot dry weight and amount of N fixed as a result of inoculation with different elite strains on soybean. In soybean experiment, it was observed that plants inoculated with RANI 22 produced significantly 60% and 56% higher nodule biomass than the uninnoculated plants in Gidan Kwano and Gurusu farm site respectively. This result is in agreement with previous rhizobia studies. For example, Ikeogu, & Nwofia reported 6 and 10%, Okereke *et al.* 14 – 100%, Egamberdiyeva *et al.* 48 – 55% increases in seed and dry matter yield due to inoculation [13, 20, 30]. This significant difference was similar to the work of Khalid *et al.* [21], where they observed that inoculation significantly increased dry weight of shoot and root, number of nodules per plant and number of pods per plant of soybean compared to control. This was also in agreement with the research carried out by Elsheikh, 1998, Okereke and Unaegbu 2006: Muhammad, 2010 [14, 25, 30]. This soybean trial indicates that some of the elite rhizobia strains stimulated improved nodulation and plant shoot biomass, which were significantly different from the control although this did not translate into increased grain yield and this was similar to the work of Osunde *et al.* [32].

The amount of N fixed by the elite strains did not show any superiority over the indigenous rhizobia of the soil in Gurusu. However, NAK 84 and RANI 22 significantly fixed more N than the control, USDA 110, USDA 532C and 60 kg N ha^{-1} in Kodoko. The range of the amount of N fixed was in the range of 3.8 - 39.9 Kg N ha^{-1} which is within the range of 5.0 -74.0 Kg N ha^{-1} obtained from previous studies [18, 32, 35].

5. Conclusion and Recommendations

The aim of this study was to identify elite strains that may stimulate increased shoot biomass, grain yield and fix more N. In general, it can be concluded that responses by the respective legume plants varied from one farm site to another. For groundnut, plants inoculated with IGB 469 produced significantly higher pod and grain yield by 30% over the uninnoculated plants in Gidan Kwano, 27% heavier shoot biomass over the control in Kodoko and SNN 343, SBG 234 and IGB 469 fixed more N than the control in Gidan Kwano. In soybean experiment, plants inoculated with RANI 22 produced significantly 60% and 56% higher nodule biomass than the uninnoculated plants in Gidan Kwano and Gurusu farm site respectively. In Kodoko and Gurusu, RACA 6 and RANI 22 produced more shoot dry biomass than the control respectively. In Kodoko farm site, NAK 84 and RANI 22 significantly fixed more N than the control, USDA 110, USDA 532C and 60 kg N ha^{-1}. The soybean result indicated that some of the elite strains stimulated increase in nodulation and shoot biomass which were significantly different from the control although this did not translate to increased grain yield indicating that inoculation still has the potential of increasing grain yield in this environment.

Considering the population of the indigenous rhizobia of the farm sites, further evaluation of the quality and quantity of these elite rhizobia strains should be done to ascertain elite strains that can effectively compete with the indigenous rhizobia in these areas.

Further research on other factors such as crop history and time of the year that may affect the population of indigenous rhizobia of the study area should be carried out to obtain more information on indigenous rhizobia in the soils around Minna. Also, the percent N in the urea fertilizer should be analyzed prior to application to check the quality of fertilizer used as the reported higher rates of fixed N by plants that received 60 kg N ha^{-1} compared to the uninnoculated plants calls into question the quality of the urea fertilizer used. Also the choice of maize as reference crop may have some effect on N fixation as the dry weight of the maize was more than the legumes. Thus further studies on legume N fixation should consider the chemical integrity of the N fertilizer used prior to application and the choice of reference crop used.

The groundnut and soybean elite strains should be considered for further studies that would involve different locations and cultivars of the legumes. The study should also call for advanced rhizobiological study that will identify the exact rhizobia strain dominating the root nodules of the legumes.

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