Soybean response to rhizobium inoculation in two Agro ecological zones of Northern Nigerian savannah

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ABSTARCT

This experiment was conducted in Kano (Sudan savanna) and Bauchi (Guinea savanna) states of Nigeria between 2015 to 2016 cropping seasons to assess microbial inoculants use for soybean production in northern Nigeria. The experiment in each location was a randomized complete block design (RCBD) with seven treatments and replicated four times. Two rhizobia inoculants and combination of treatments were tested on soybean (TGX 1835) in the two agro-ecological zones to monitor their performance and their ability to establish symbiotic relationship and nodulate soybean. The treatments were; Legume fix, Alosca, nitrogen, cattle manure, Legume fix + cattle manure, Alosca + cattle manure and control. Most probable number (MPN) method was used to assess the number of rhizobia cells in the inoculants used for the field experiment. Prior to this, the soils in the study locations were tested for physico-chemical properties and the population count of the indigenous bacteria. The results indicated that Legume fix and Alosca influence the yield of soybean in the study area by giving high yield compared to the control treatments.

Key words: Inoculants, soybean nodules, rhizobia, cattle manure, legfume fix, alosca and symbiotic

1. Introduction

Soybeans is like other legumes type of plant possess the potential natural capacity to fix its nitrogen through biological nitrogen fixation. Compatibility is necessary between the rhizobia and the host legume for the successful nodulation to occur. Legumes are positioned as the third largest group of angiosperm plants and also the second largest group of food for human as well as feed for animals in the world (Akinbamijo *et al*., 2018). It includes various food crops like alfalfa, beans, peanut, cowpea, faba beans, bambara groundnut, clover, chick pea, soybean and so on. Thus, when growing newly introduced legume to an area there is tendency for nodulation to fail due to the presence of native rhizobia which may not be compatible to the crop, so appropriate rhizobia culture is necessary to be applied (Solomon *et al.*, 2012). Several other factors can influence the nitrogen fixation capability of legumes. These may include: type of bacteria that may be present in the soil (native bacteria), the nitrogen content of the soil, the type of legume and its compatibility with bacteria, diseases and favorable pH (Keyser and Li, 1992). Avery important way of closing yield gap is the usage of Artificial inoculation of legumes with suitable strain of rhizobium (Tairo and Ndakidemi, 2013b). Inoculation was observed to have effect on improved yield in some legume plants. (Albareda *et al*., 2009)

Through the ability of legumes to form symbiotic association with rhizobia that can fix atmospheric nitrogen into plant usable form, it helps in the sustenance of crop production and also reflation the soil with nitrogen. This can be achieved through the knowledge on how much nitrogen is being fixed by the legume and the influence of soil management practice in this regard (Peoples *et al*.,1989). In recent days most of the researches concerning biological nitrogen fixation focus more on the bacterial side than the host legume plant, despite the fact that only under extreme condition that bacteria pose a serious limitation in biological nitrogen fixation. This needs to be given focus attention in the developing countries where other factors hinder crop production than bacteria itself (Thomas and Vincent, 2012). The main objective of this study was to investigate the rhizobia inoculants performance on soybean in two agroecological zones of northern Nigeria savannahs (Guinea savannah and Sudan savannah).

2. Materials and Methods

2.1 Study site and soil characteristics

This study was conducted at 2 locations: Kano University Science and Technology Research farm at Bagauda (Sudan savannah) located at latitude 11º 37. 409' N and longitude 08º 22. 994' E, altitude 481 meters (or about 1580 feet) above sea level and Abubakar Tafawa Balewa University Research farm at Gubi (Guinea savannah) located on latitude 10°27. 985' N and longitude 9°49.768' E. The area is situated at about 666.5 m above sea level. The study was conducted during 2015 cropping season.

Initial soil sample was done by collecting twelve core soil samples from depth of 0-15 cm from each block from the two experimental sites following a 'W' design before planting. Composite sample was obtained after careful mix of the soil and air dried before sieving through a 2 mm mesh sieve. The collected samples were leveled and packed in a clean polythene bags before being taken to the laboratory for selected chemical and physical analyses. Particle size distribution of soil sampled was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was determined according to Page *et al*., 1982. The modified Walkey and Black procedure as described by Nelson and Sommers (1982) was used to determine organic carbon. The method involved chemical or wet oxidation then followed by the measurement of expelled CO_2. Soil total nitrogen content was determined using the Macro - kjeldahl method. The method involved digestion, distillation and titration as described by Bremner and Mulvaney (1982). Available phosphorus was determined through Bray No 1 method to extract the readily acid soluble forms of phosphorus from the soil as described by Olsen and Sommers (1982). Exchangeable bases (K, Ca, Mg and Na) were determined by 1.0 M ammonium acetate (NH4OAc) extract. The exchangeable acidity was determined using titration method as described by Mclean (1965).

2.2 Field layout and experimental treatments

After clearing and land preparations, experimental plots were demarcated. Each plot size 4 by 4.5M. There were seven plots replicated four times. The inter raw spacing was 0.75m. Soybean seed was sawn 3 seeds per hole later thinned to two per hole after emergence. The distance between stands was 10 cm in between. Wedding was done with hoe regularly. No any herbicide was applied throughout the growing period. Soybean seeds were sown during 2015 cropping season at Sudan and Guinea savannah agro ecological zones.

2.3 Estimation of Indigenous Rhizobia population

The most probable number method was used to estimate the population of native rhizobia from the study area as described by (Somasegaran and Hoben, 1994). The indigenous population of rhizobia from the experimental sites were shown in Table 1.

Composite soils from the experimental sites were collected and used to inoculate soybean in a growth pouch using dilution $^{-}1$ to $^{-}5$ and replicated five times in each case. After successful germination, the seedlings were carefully placed into the growth pouch with the radicle pointed down. A nutrient solution free of N was prepared and addition was done regularly. A serial dilution was prepared by taking 100 g of the soil and diluting it with 400 mL of distilled water. A fivefold serial dilution was made $^{-}1$ to $^{-}5$, growth pouches were replicated five times and set in green house and inoculated with 1 mL dilution. The plants were observed for 4 weeks. There after the nodules were observed and counted. Number of rhizobia were determined using the following formula:

$$X = \frac{m \times d}{v}$$

m = likely number from the MPN table for the lowest dilution of the series

d = lowest dilution (first unit)

v = volume of aliquot applied to plant

2.4 Grain yield and harvest index

After harvesting the plants at physiological maturity, the grains were oven - dried at 60 °C for 72 hours. The grain dry weights for each of the plot were then recorded and use to determine grain yield per hectare per plot (Okogun *et al*., 2005).

$$H\,I = \frac{PY}{TY}$$

Where : HI = harvest index

TY = total yield

PY= pod yield

2.5 Inoculation

Soybean seed used for the experiment was TGX1835. The seeds were inoculated with legume fix and alosca inoculants based on the recommendation of manufacturers (50g per 1 kg of seed). The seeds were placed in a clean container, gum arabic was added with little quantity of water before pouring the inoculants and shaken gently to ensure stickiness with the seeds. The seeds were allowed to dry a little under the shade before planting. Treatments were: T1= Legume fix, T2= alosca, T3= nitrogen (Urea 50 kg N ha $^{-1}$, T4= Cattle manure (4 tonnes /ha), T5= Legumefix + cattle manure, T6 = alosca + cattle manure, T7 = Control.

2.6 Nodule dry weight and nodule effectiveness

Ten plant samples were randomly selected and then carefully uprooted from the two inner rows of each plot at peak flowering stage to assess nodulation. A spade was used to uproot plants from the soil. Care was taken to collect all the nodules including the detached ones and kept inside the ziplock bags, labelled and taken to the laboratory where they were carefully washed and counted. The nodules of each of the treatments were subjected to oven drying at 75 ^{o}C for 48 hours and the weight was recorded to obtain the nodule dry weight for each treatment.

Ten nodules were randomly selected and sharp razor blade was used to cut open the nodules longitudinally to examine their color and appearance for nodule effectiveness. Nodules that appeared reddish or pink in color were regarded as effective ones. Percent effective nodules was calculated as the ratio of active nodules to total number of nodules.

3. Results

3.1 Soil characterization and the indigenous rhizobia populations

The result for soil characterization and indigenous rhizobia population was presented in Table 1.

The soil was sandy loam in the textural class. Total nitrogen content was 0.11 % in both locations. Available phosphorus values recorded on Sudan and Guinea savannahs were 8.05 mg kg $^{-}$1 and 4.03 mg kg $^{-1}$ respectively. The soil pH (1:1 H_2O) values of Sudan savannah soil and Guinea savannah soil were 5.8 and 6.0 respectively. The exchangeable potassium values were found to be 0.02 cmol$_{(+)}$ kg^{-1} for Sudan savannah soil and 0.24 cmol$_{(+)}$ kg^{-1} for Guinea savannah soils. Soils of Sudan savannah have inherent K value below the critical level of 0.15 cmol $_{+}$ kg^{-1}. However, K for Guinea savannah was 0.24 cmol$_{(+)}$ kg^{-1} which is slightly above the critical limit value. The organic carbon values of Sudan and Guinea savannahs soils were found to be 0.41% and 0.37% respectively. The IRP were found to be 1.02 x 10^1 cells g^{-1} of soil and 2.20 x 10^1 cells g^{-1} for Sudan and Guinea savannahs respectively.

Table 1. Physico chemical properties and indigenous rhizobia counts of experimental sites (initial 2015)

Parameters	**Sudan savannah soils (Bagauda location)**	**Guinea savannah soils (Gubi location)**
pH (1:2.5 H_2O)	5.90	6.50
pH (0.1 M $CaCl_2$)	4.70	5.10
Organic carbon (%)	0.41	0.37
Total nitrogen (%)	0.11	0.11
Available P (mg kg $^{-1}$)	8.05	4.03
Exchangeable bases		
Ca^{2+}	5.20	3.63
Mg^{2+}	1.04	0.43
K^{+}	0.04	0.11
Na^{+}	0.08	0.12
Total exchangeable acidity		
(Al^{3+} +H^{+}) (cmol(+) kg^{-}1)	0.8	0.4
ECEC	7.6	5.3
Sand (%)	66.00	68.00
Silt (%)	22.00	18.00
Clay (%)	12.00	14.00
Texture	Sandy loam	Sandy loam
IRP(cells g^{-1} of soil)	1.02 10^1	2.20 x 10^1

IRP - Indigenous rhizobia population

3.2 Nodule number and nodule dry weight of soybean

The results of nodule number and nodule dry weight for soybean at Sudan and Guinea savannahs are presented in Table 2. Soil in the two locations responded to treatments. The mean values of treatments for nodule number in Sudan savannah were significantly ($P \leq 0.001$) different for some treatments. The treatment combination legume fix + cattle manure gave the highest nodule number which is statistically different with control which recorded the least value of 23. Result further showed that cattle manure and legume fix also gave the high value of nodule number. Alosca gave the value higher than control.

At Guinea savannah significant difference ($P \leq 0.001$) was observed for soybean nodule number in some treatments. Treatment combination of alosca + cattle manure was the highest followed by legume fix + cattle manure. Here the 50 kg N ha^{-1} treatment recorded the least nodule number although not significantly different with control. Treatments combination of inoculants with cattle manure i.e. alosca + cattle manure and legume fix + cattle manure gave highest nodule of 40 and 30 respectively. All other treatments were higher than the control.

Table 2. Nodule number, nodule effectiveness and nodule dry matter weight of soybean

Treatments	Nodule number (plant^{-1})		Nodule effectiveness (%)		Nodule dry weight (kg ha^{-1})	
	Sudan savannah	Guinea savannah	Sudan savannah	Guinea savannah	Sudan savannah	Guinea savannah
Legume fix	44.98 ^{ab}	28.15 ^{bc}	85 ^{a}	85 ^{a}	3.87 ^{abc}	5.42 ^{a}
Alosca	35.13 ^{cd}	29.50 ^{b}	70 ^{a}	77 ^{a}	3.54 ^{bcd}	2.96 ^{b}
Nitrogen (50 kg N ha^{1})	30.55 ^{d}	16.38 ^{d}	32 ^{b}	25 ^{c}	2.49 ^{d}	2.21 ^{b}
Cattle manure	40.98 ^{bc}	26.55 ^{bc}	35 ^{b}	45 ^{b}	5.12 ^{a}	5.37 ^{a}
Legume fix + cattle manure	48.40 ^{a}	30.38 ^{b}	80 ^{a}	87 ^{a}	4.92 ^{ab}	5.49 ^{a}
Alosca + cattle manure	40.40 ^{bc}	39.32 ^{a}	77 ^{a}	80 ^{a}	4.4 ^{ab}	5.29 ^{a}
Control	23.30 ^{e}	22.12 ^{cd}	37 ^{b}	27 ^{bc}	2.58 ^{d}	2.17 ^{b}
F pr.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lsd (5%)	6.6	4.2	1.72	1.92	1.33	1.23
CV (%)	4.1	1.2	9.0	4.0	4.0	2.5

For nodule dry weight soybean at Sudan savannah, 50 kg N ha^{-1} treatment recorded the least value 2.49 (kg ha^{-1}) and differs significantly ($P \leq 0.001$) from all other treatments except alosca and control. Cattle manure treatment which has highest value of 5.12 were similar to treatment combination of legume fix + cattle manure, alosca + cattle manure and legume fix. The result of nodule dry weight soybean at Guinea savannah showed that significant difference ($P \leq 0.001$) exists between treatments. Highest value of 5.49 (kg ha^{-1}) was recorded by treatment combination of legume fix + cattle manure, but not statistically different with alosca + cattle manure, cattle manure and legume fix alone. Least value of nodule dry weight was observed in Control treatment which does not differ significantly with 50 kgN ha^{-1} and alosca treatments.

3.3 Grain yield and Harvest index

Table 3. Showed the grain yield and harvest index of soybean at Sudan savannah location. The results showed significant differences (P≤ 0.001) between some treatments. In Sudan savannah, yield increase of 62, 30, 36 and 70% were recorded for legume fix, alosca, legume fix + cattle manure and alosca + cattle manure respectively over the control. Alosca also differed significantly from all other treatments. Harvest index results at Sudan savannah showed that legume fix gave the highest harvest index (0.75) which was significantly different (P≤ 0.001) from all other rest treatments.

Table 3. Grain yield and harvest index of soybean at Sudan savannah

Treatments	Grain yield (kg ha^{-1})	Harvest index
Legume fix	1366 ^{a}	0.75 ^{a}
Alosca	1099 ^{b}	0.45 ^{c}
Nitrogen (50 kg N ha^{-1})	873 ^{c}	0.53 ^{bc}
Cattle manure	891 ^{c}	0.27 ^{d}
Legume fix + cattle manure	1145 ^{b}	0.59 ^{b}
Alosca + cattle manure	1436 ^{a}	0.47 ^{bc}
Control	843 ^{c}	0.27 ^{d}
F pr.	< 0.001	< 0.001
Lsd (5%)	167.3	0.135
CV (%)	7.8	9.2

Result of the effect of inoculation on grain yield and harvest index of soybean at Guinea savannah is presented in Table 4. Results showed that some treatments differed significantly (P≤ 0.001). Legume fix recorded the highest grain yield value (1342 kg ha $^{-1}$). Yield increases of 51, 41, 33, 26 and 21% were observed over the control for legume fix, legume fix + cattle manure, cattle manure, alosca + cattle manure and alosca respectively. Grain yield value for 50 kg N ha^{-1} (997 kg ha $^{-1}$) was not significantly different from the control (890 kg ha $^{-1}$). The harvest index for Guinea savannah was generally higher than that of Sudan savannah (Table 4.11). Legume fix recorded the highest harvest index (0.82 kg ha^{-1}) which differed significantly from all other treatments except the 50 kg N ha^{-1} treatment. The control produced the least value of 0.52 kg ha $^{-1}$. Legume fix, 50 kg N ha^{-1} and cattle manure recorded increased harvest index values of 57, 42 and 21% respectively over the control. Legume fix + cattle manure, alosca + cattle manure, cattle manure and alosca were not significantly different from the control (P= 0.012).

Table 4. Grain yield and harvest index of soybean at Guinea savannah

Treatments	Grain yield (kg ha^{-1})	Harvest index
Legume fix	1342 ^{a}	0.82 ^{a}
Alosca	1082 ^{bc}	0.53 ^{c}
Nitrogen (50 kg N ha^{-1})	997 ^{cd}	0.74 a^{b}
Cattle manure	1188 ^{ab}	0.63 ^{bc}
Legume fix + cattle manure	1257 ^{ab}	0.55 ^{c}
Alosca + cattle manure	1125 ^{bc}	0.59 ^{bc}
Control	890 ^{d}	0.52 ^{c}
F pr.	< 0.001	0.012
Lsd (5%)	180.1	0.17
CV (%)	6.1	11.6

4. Discussion

From the result of initial soil test, it was shown that the soil texture was sandy loam in both Sudan and Guinea savannahs experimental fields. However, soil at Sudan savannah was moderately acidic while that of Guinea savannah was slightly acidic (Landon, 2014). Soil organic carbon was low (< 1%) in both study locations (Landon, 2014). This low organic carbon can be attributed to continuous cultivation of the area and limited addition of organic materials as a typical characteristic of savannah soil as reported by Ogundare *et al*. (2012). The available N and P content of the soil in the two locations is considered to be low the critical levels (N <0.10% and P < 10 mg kg $^{-}$1) (Landon, 2014). The K values of Sudan savannah soil was lower than the soil in Guinea savannah and this could be as a result of the parent material reach in K. Therefore, response to K may not be observed in such soil. In general, the soils in the two study locations have low inherent fertility (Singh *et al*., 2011). The indigenous rhizobia population (IRP) was found to be low in both locations. The IRP were found to be 1.02×10^{1} cells g^{-1} of soil and 2.20 x 10^1 cells g^{-1} of soil at Sudan and Guinea savannahs. This means that the soil in the two study locations did not contain enough indigenous rhizobia population. There is tendency for the soils to respond to inoculation. According to Sanginga *et al*. (1996) ; Houngnandan *et al*. (2000) and Zoundji *et al*. (2015), response of soil to inoculation is likely to happen when the population of indigenous rhizobia is between 5 to 10 cells g^{-1} soil.

Nodule is the major criterion for assessing biological nitrogen fixation by *Bradyrhizobium* and therefore an important index for fixation potential (Singleton and Tavares, 1986a Ampomah *et al*., 2008; Workalemahu, 2009; Argaw, 2014;). Legume fix inoculation gave the highest number of soybean nodule number compared to alosca. Likewise, percentage nodule effectiveness under soybean field showed positive response to inoculation. Plots treated with 50 kg N ha^{-1} always showing low percentage effectiveness this could probably be due to the fact that the bacteria will be inactive in fixation under 50 kg N ha^{-1} sufficient condition. The efficacy of legume fix over alosca could probably be due to the presence of larger number of stains than in alosca (Tabl. The strains could probably be more effective than those of alosca. Several number of researchers reported a significant increase in nodule number due to inoculation with appropriate *Bradyrhizobium* (Martins *et al.*, 2003; Osunde *et al*., 2003; Kumaga and Ofori, 2004). Yakubu *et al*. (2010) while working with cowpea in North Eastern Nigeria confirmed that inoculation with *Rhizobium* increased nodule number of cowpea by more than 30% compared to the control. In this current study, the nodule dry weight was significantly higher than the control. The result is in agreement with that of Santos *et al*. (2011) and Solomon *et al*. (2012) who observed that rhizobia inoculation significantly increased nodule dry weight. The cropping history of the two experimental sites suggested that soybean had not been grown, hence it was expected that inoculation will work well. This is in agreement with finding of Asei *et al*. (2015) who opined that compatible rhizobia population is low in soil where soybean has not been grown for long and hence inoculation with superior strains of rhizobia is necessary under this condition if good nodulation and subsequent yield boost is to be achieved. Other workers stated that, soybean grown in Africa was believed to be capable by forming effective nodules with indigenous *Bradyrhizobium* species in some soils where soybean has been grown over a long period of time (Maingi *et al*., 2006; Yoseph *et al*., 2017). The high number of nodules in legume fix and alosca treatments is in line with claim of many researchers that inoculation results in higher number of nodules than the control treatments (Okereke *et a*l., 2004; Tahir *et al*., 2009; Bekere and Hailemariam, 2012). This confirm the null hypothesis of this study that inoculation will enhance nodulation performance of soybean. This positive response is in line with reports of several workers who reported on the successful nodulation in legumes when compatible rhizobia were used in inoculation (Kishinevsky *et al*., 1987; Elsheikh and Wood, 1995; Sanginga e*t al*., 1996; Burdass, 2002; Gwata *et al*., 2003; Singh and Usha, 2003; Abaidoo *et al*., 2007; Ndusha *et al*., 2017; Kumar *et al*., 2018).

The low number of nodules in 50 kg N ha^{-1} treatment was not surprising because of the high amount of nitrogen has been reported to inhibit nodulation process (Unimke *et al*., 2016). Generally, the nodule numbers of soybean at Guinea savannah was lower as compared to Sudan savannah. This could be attributed to variability in soil and climate in the two locations as well as the nature of rhizobia population. This corroborate with findings of group of researchers while working with soybean in northern Nigerian savannah who opined that yield and general performance of soybean was affected by soil as well as the environmental and management factors (Ronner *et al*., 2016). Another reason could be that the strains in the inoculants thrives better in Soils of Sudan savannah. Nodule dry weight follows the same trend with nodule number. Inoculation affects nodule dry weight significantly compared to control treatments.

Grain yield and harvest index result for soybean (Table 4) has indicated the inoculants treatments have significantly affected the yield both at Sudan and Guinea savannahs locations. Alosca + cattle manure treatments produced highest yield followed by alosca alone. The highest harvest index was also recorded under inoculated treatments. This result is expected due to the fact that soybean was a new crop to the study locations, it was therefore anticipated that the inoculants will establish a healthy symbiotic kind of relationship with soybean to fix required nitrogen and also influence yield increment. The result tallied with finding of many workers that established that inoculation influences the yield especially in an area where new legume was introduced (Martins *et al*., 2003; Unkovich *et al*., 2008; Osunde. *et al*., 2003; Kolapo, 2011; Meghvansi, Prasad and Mahna, 2010; Osman, 2011; Binang *et al*., 2013; Mutuma *et al*., 2014; *Asei et al*., 2015; Rahim *et al*., 2017). From the interview conducted with farmers, it was discovered that soybean was a new crop at the study location. It is necessary to inoculate when new crop is introduced to an area to achieve the yield potential (Zimmer *et al*., 2016). This was also confirmed by some workers in Kenya who opined that commercial products rhizobium inoculants can supply effective strains for use in areas where soybean is being introduced as a new crop (Thuita *et al*., 2012). The low grain yield obtained under 50 kg N ha-^{1} treatment is contrary to the result by Tahir *et al*. (2009) who found out that application of 25 kg N in combination with TSP fertilizer resulted in increase of soybean grain yield. However, this reduction of yield under 50 kg N ha^{-1} could be linked to the shortfall of water during the blooming stage around September (appendix 1). It was reported that water stress could result in yield decrease under 50 kg N ha^{-1} fertilization (Moser *et al*., 2006).

5. Conclusion

Based on the findings of this study, it could be concluded that the application of rhizobium inoculant enhanced soybean nodulation and consequent yield increment in both Sudan and Guinea savannah agro ecological zones of Nigeria. However, it was observed that generally legume fix performed better than alosca. Therefore, cultivation of soybean inoculated with compatible rhizobia strains will improve soil N fertility and in turns reduces the usage of high cost mineral nitrogen. This will result in the enhancement of farmers economic wellbeing in the area.

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Appendix 1. Monthly cumulative rainfall distribution at Sudan and Guinea savannahs