Effect Of Rhizobia Inoculation On Nodulation And Nitrogen Fixation Of Soybean Cultivars In Sudan Savanna Of Nigeria

M. ISMAILA
D. D. DAN'AZUMI
Department of Agricultural Technology,
Yobe State College of Agriculture Gujba,
P.M.B. 1104, Damaturu, Nigeria.
*Corresponding author: ismimoh@gmail.com

M. BABAGANA
Department of Animal Health and Production,
Yobe State College Of Agriculture Gujba, P.M.B 1104, Damaturu, Nigeria.

M. J. JIBRIN
Faculty of Agriculture, Department of Soil Science, Bayero University, P.M.B. 3011 Kano, Kano Nigeria.

A. L. NGALA.
Faculty of Agriculture,
Department of Soil Science,
University of Maiduguri, Nigeria.

Abstract- **A field experiment has been conducted to study the effect of rhizobia inoculation and nitrogen fixation of soybean cultivars. The experiment consisted of testing ten cultivars under bradyrhizobium inoculation and uninoculation conditions in a split plot design and replicated four times, with inoculation in the main plot and the varieties in the sub plot level. The parameters determined were initial physico-chemical properties of the soil, number of nodules and nodule score, and biological nitrogen fixation. The result indicated that the soil was low in fertility with low organic matter, total N, available P and Sulphur. Nodule score and number of ineffective nodules were significantly affected as there were significant differences between the inoculated and uninoculated treatments. Similarly, varietal effect on number of effective and ineffective nodules and number of nodules were significant ($P<0.05$), but nodule score was not significantly affected. The highest number of effective nodules and number of nodules were observed in TGX 1945 and TGX 1935–3F, respectively, while the lowest was with SC–SAGA. Highest N_2 fixed (150 Kg N ha^{-1}), equivalents to 63% was observed with TGX 1935-3F, while the lowest (100 Kg N ha^{-1}), equivalent to 55% N was with SC-SAGA. Generally, the proportion of plant N derived from atmosphere and the estimate of N fixed were not significantly ($P>0.05$) affected by inoculation and varieties.**

Keywords—Inoculation, Nodulation, Nitrogen Fixation, Soybean, Kano, Nigeria

I. INTRODUCTION

The increasing cost of fertilizers and their impact on the environment have forced people to look for other alternative sources of plant nutrients. In this regard, nitrogen fixation, which is a process by which elemental atmospheric nitrogen is changed to organic forms by both symbiotic and asymbiotic microorganisms in soil,
has drawn much attention. The symbiotic nitrogen fixation is used to maximum advantage in case of leguminous crops. There is no doubt that specificity exists between rhizobial strain and the legume, and compatibility between the two is essential for successful nodulation. This necessitates using specific cultures for different legumes. When growing new legume specie on a soil, it is necessary that the appropriate rhizobial culture be applied [1]. Use of rhizobium inoculums in the establishment of legumes has been widely recognized, especially in areas where indigenous nodulation has been found to be inadequate. The benefits derived by the use of rhizobium inoculants show that a quite good deal of money can be saved by marginal farmers by using quality tested inoculants on the farm. Further, it has been reported that the legume crops enrich the fertility of the soil. Rhizobial inoculation to seeds is well studied and exploitation of this beneficial nitrogen fixing root nodule symbiosis represents a hallmark of successfully applied agricultural microbiology [2]. Therefore, Biological nitrogen fixation plays an essential role in crop establishment and yield, since no N fertilizer is applied and it fulfills most of plants need for nitrogen [3], [4]. Interest in BNF has focused on the symbiotic systems of leguminous plants and rhizobia because these associations have the greatest quantitative impact on the nitrogen cycle. Deficiency in mineral N often limits plant growth and as such, symbiotic relationships have evolved between legume plants and a variety of N_2-fixing organisms. The symbiotically fixed N_2 by the association between rhizobium species and the legumes represents a renewable source of N for agriculture. Values estimated for various legume crops and pasture species are often impressive, commonly falling in the range of 200 to 300 kg N ha^{-1} per year. This underlines the significance of

rhizobium and legume symbiosis as a major contributor to BNF [5], [6].

Increased attention is being paid to improving the N_2 fixation of promiscuous soybeans in an attempt to develop sustainable cropping systems in moist savanna [7]. There is however dearth of reliable information on response of promiscuous soybean varieties to inoculation in the Sudan savanna zone, hence, this paper aims at investigating the response of promiscuous soybean varieties to inoculant.

II. MATERIALS AND METHODS

A. Experimental site description

The experiment was conducted at Bayero University, Kano (BUK) Teaching and Research Farm (latitude 12^O 58^{1}N, longitude 8^O 25^{1}E) in the Sudan savanna agro-ecology. The location was characterized by two seasons: a wet season (May to September) and dry season (October to April). The amount of rainfall received during 2011 wet season of the field trial was 1041.80 mm. The daily minimum and maximum temperatures during the period ranged from 18 to 34 ^{O}C, respectively.

B. Field experimental design

Field trial was conducted in the year 2011 rainy season, where nine improved soybean varieties viz.: TGX 1835-10E, TGX 1904-6F, TGX 1935-3F, TGX 1951-3E, TGX 1448-2E, TGX 1955-4E, TGX 1485-1D, TGX 1987-62F, TGX 1945-3E, and one local variety SC SAGA, natural fallow and maize were grown under two levels of *bradyrhizobium* inoculation conditions (inoculated and un-inoculated). The treatments were laid out in a split plot design in four replications, with inoculation in the main plots and varieties in the sub plots.

C. Agronomic practice

Before the establishment of the experiment, the land was ploughed and harrowed to a fine tilt and ridged at 0.75m apart. Later the land was marked into plots and replications. An alley of 1.5 m and 2.0 m were left between plots and replications, respectively. The gross sub plot size consisted of 6 rows, 0.75 m apart and 3 m long. Each plot size measured 4.5 m broad x 3.0 m long (13.5 m^2); while net plot was 3 m wide x 1.5 m (4.5 m^2). Sowing of soybean was done at 3 seeds per hole at about 0.75 m apart between rows and 0.10 m between stands. Single Super Phosphate (SSP) was applied at a rate of 20 kg per hectare at planting. The fertilizer was banded 0.10 m away from the planting line, in a 0.02 m deep trench and covered after application. Weeds were controlled manually using hoe as often and when necessary. Cases of pests and diseases were not observed throughout the trial.

D. Soil analysis

Before the establishment of the trials, soil samples were collected randomly from 0-15 cm using auger for determination of physico-chemical properties. Each soil sample was a composite of five sub-samples. The collected soil samples were properly labeled and stored in polythene bags and taken to the laboratory, air-dried, sieved using 2 mm mesh sieve and analyzed. Routine soil analysis was carried out to determine particle size distribution by hydrometer method as outlined by [8]; soil pH was determined Potentiometric as described by [9]; Organic carbon was determined by Walkely-Black method as outlined by [8]; total N was determined by modified Kjeldahl method as described by [10]. Others are available P determined using Mehlich-3 extraction procedures as described by [10]; Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1 N ammonium acetate [11]; available Sulphur was determined by turbidimetric method [12], using extraction solution of 1 M KCl and exchangeable bases (Ca, Mg, K and Na) were extracted with 1N Ammonium acetate (NH_4OA_C) while Ca and Mg were determined using atomic absorption spectrophotometer; K and Na were determined using flame photometer [8].

E. Nodulation analysis

Destructive sampling for nodule counts and nodule effectiveness were done at full pod according to standard procedures [13]. Five plants were sampled randomly from the net plot at full pod. The whole plant was carefully uprooted using a spade so as to obtain intact roots and nodules for nodulation parameters. Uprooting was done by exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with water. The same five plants from each plot were used to rate nodulation and to record number of nodules per plant.

- Nodule score

Nodule score was obtained using 0 to 5 ranking system. This particular system scores nodulation from 0 to 5, the nodule score is determined by the number of effective nodules in the crown-root zone (regarded as the region up to 5 cm below the first lateral roots) and elsewhere on the root system [14]. The scores from all plants are added and then divided by the number of plants to obtain a mean nodule score. A mean nodule score were determined as follows:

(i) 4–5 represents excellent nodulation; excellent potential for N_2 fixation,

(ii) 3–4 indicates good nodulation; good potential for N_2 fixation,

(iii) 2–3 represents fair nodulation; N_2 fixation may not be sufficient to supply the N demand of the crop,

(iv) 0–2 indicates poor nodulation and probably little or no N_2 fixation.

- Effective and ineffective nodule

A maximum of ten nodules were stripped off the crown and main roots of each sampled plant and sliced into halves to expose the center where specific nodule colours were observed. Effective nodules were determined by red, pink and brown colours in the center whereas yellow, green and other colours depicted ineffectiveness [15].

F. Quantification of biological nitrogen fixation

Quantification of the amount of N_2 fixed was carried out by the N difference method [13]. In the N-difference method, the difference between total plant nitrogen of a N_2- fixing legume and a control crop (non- N_2- fixing) was considered to be nitrogen that has been fixed biologically. The quantity (Q) of biologically fixed N_2 was calculated as follows:

[Q = N yield (legumes) − N yield (control)].

Where:

Q (kg ha^{-1}) = Quantity of the biologically fixed nitrogen

N yield [legumes] (kg ha^{-1}) = Nitrogen yield of legumes

N yield [control] (kg ha^{-1}) = Nitrogen yield of a non-fixing plant.

G. Data analysis

Data obtained were subjected to

statistical analysis using the analysis of variance

[16]. Means that were significantly different were separated using the Least Significant Difference (L.S.D.) as reported by [17].

III. RESULTS AND DISCUSSION

The physico-chemical properties of the soil (0-15 cm) of the trial site before establishment of the trials are presented in Table 1. The soil texture was characterized as sandy loam. The soil pH (H_2O) was slightly acidic while organic carbon, total N, available phosphorus, and Sulphur contents were all low. The cation exchange capacity (CEC) and exchangeable bases were also in the low fertility class (less than 5.0 and 50 Cmol/kg, respectively) [18]. Thus, improvement in soil chemical properties is expected.

Table 1: Initial Physico-chemical properties of surface soil of the experimental site

Soil property	Fertility Class[18]	
Particle size distribution (g kg^{-1})		
Clay	170	-
Silt	40	-
Sand	790	-
Textural class	Sandy loam	-
Exchangeable bases (cmol kg^{-1})		
Ca	0.75	Low
Mg	0.50	Low
K	0.07	Low
Na	0.33	Low
CEC (cmol kg^{-1})	5.00	Low
pH in H_2O (1:2.5)	6.70	Slightly acidic
Organic Carbon (g kg^{-1})	9.5	Low
Total Nitrogen (g kg^{-1})	3.6	Low
Available Phosphorus (g kg^{-1})	10.1	Low
Available Sulphur (µg /g)	0.43	Low

Source: Laboratory analysis

The effects of inoculation and variety on number of nodules and nodule score at full podding of soybean are presented in Table 2. There was significant ($P<0.05$) difference between inoculated plants and uninoculated plants with regard to nodule score and number of ineffective nodules. The effect of varieties on nodule score was not significant but there was significant ($P<0.05$) difference between varieties in number of effective, ineffective nodules and number of nodules. The highest number of effective nodules was observed in TGX 1945 which was at par with TGX 1485-1D, while the lowest was with SC–SAGA. However, TGX 1835–10E was observed to have the highest number of ineffective nodules, though at par with TGX 1935–3F. Highest number of nodules was observed in TGX 1935–3F but this was statistically similar to TGX 1835-10E and TGX 1904–6F, while least number of nodules was observed in SC-SAGA. Generally, inoculation produced significant ($P<0.05$) increase in nodule score by stimulating the formation of nodules by roots of soybean. This is in agreement with the findings of [19]. [20], reported that inoculation of soybean with *Bradyrhizobium japonicum* strain resulted in a significant increase in nodule score on the Nitisol of Bako. However, the number of effective nodule was found to be insignificant. This could be associated with the time of sampling the nodules, which was carried out at full podding of the soybean. Reports have shown that most of the nodules at this period (full podding) might have senesced [21]. This could probably explain why the numbers of effective nodules were insignificant. Contrary to this finding, larger response to inoculation and higher number of nodules per plant in comparison to uninoculated treatments in a field that has no soybean cropping history was reported by other workers [22], [23], [24]. However similar works have suggested that inoculation does not always elicit or enhance nodulation. [25], reported that improve soybean variety (TGX 1448-2E) did not respond to

inoculation in terms of nodule production in the Nigeria's moist savanna zone. The interaction

between inoculation and variety did not significantly (P>0.05) affect nodulation.

Table 2: Effects of Inoculation and variety on Nodulation of Soybean

Treatment	Nodule score	Effective Nodule	Ineffective Nodule	Nodules/ plant
Inoculation (I)				
Inoculated	4.08[a]	41[a]	179[a]	220[a]
Uninoculated	2.84[b]	40[a]	137[b]	172[a]
SE ±	0.09	2.0	7.0	10
Varieties (V)				
TGX 1835-10E	3.85[a]	32[cd]	190[a]	223[a]
TGX 1904-6F	3.47[a]	46[ab]	174[ab]	221[a]
TGX 1935-3F	3.17[a]	39[bc]	185[a]	224[a]
TGX 1951-3E	3.12[a]	47[ab]	158[ab]	205[a]
TGX 1448-2E	3.25[a]	38[bc]	160[ab]	199[ab]
TGX 1955-4F	3.70[a]	39[bc]	177[ab]	217[a]
TGX 1485-1D	3.27[a]	47[ab]	142[bc]	190[ab]
TGX 1987-62F	3.77[a]	36[cd]	179[a]	216[a]
TGX 1945	3.80[a]	49[a]	119[cd]	169[b]
SC-SAGA	3.17[a]	28[d]	94[d]	122[c]
SE ±	0.36	4.0	17	18
Interaction				
I x V	NS	NS	NS	NS

Means followed by the same letter(s) within a treatment column are not significantly different at P < 0.05; NS= Not significant

The effects of inoculation and varieties on biological nitrogen fixation by soybean are shown in Table 3. The proportion of plant N derived from atmosphere and the estimate of N fixed were not significantly (P>0.05) affected by inoculation and varieties. It was observed that the highest N_2 fixed (150 Kg N ha^{-1}), equivalents to 63% was by TGX 1935-3F while lowest (100 Kg N ha^{-1}), equivalent to 55% N was by SC-SAGA. The amount of N_2 fixed and % Ndfa for the inoculation was higher than that of the uninoculated (control). This nevertheless indicates higher numbers of rhizobia can effectively increase the amount of N_2 fixed and % Ndfa. This is in agreement with [26] who reported increase in amount of BNF due to inoculation. The combination of inputs (interactions) failed to increase the amount of N fixed and % Ndfa. This finding is in contrast to the findings of [27], who reported that a specific combination of soybean genotype with rhizobium strains resulting in many fold increase in the amount of N_2 fixed. Estimates of the proportion of plant N derived from atmosphere (Ndfa) and the amount of N_2 fixed by legumes varieties in moist savanna vary widely [28]. Although not statistically significant, differences between the varieties in the amount of N fixed could be discerned. Reference [28] in their work conducted in moist savanna of Nigeria reported proportion of N derived from promiscuous soybean fixation to be in the range of 26-76%, equivalent to 31-110 kg N ha^{-1}. Other reports estimated N fixed by promiscuous soybean in the range of 26-64%, representing 24-168% kg N ha^{-1} [7]. However, this work reported estimates of N derived by promiscuous soybean in the range of 53-64%, representing 99-150 kg N ha^{-1}. This corroborating the earlier findings that inoculation improves biological nitrogen fixation.

Table 3: Effects of Inoculation and variety on nitrogen fixation by soybean

Treatments	Ndfa (%)	N_2 fixed (Kg ha^{-1})
Inoculation (I)		
Inoculated	64[a]	140[a]
Uninoculated	60[a]	122[a]
SE ±	4.3	24.3
Varieties (V)		
TGX 1835-10E	64[a]	143[a]
TGX 1904-6F	60[a]	108[a]
TGX 1935-3F	63[a]	150[a]
TGX 1951-3E	62[a]	134[a]
TGX 1448-2E	56[a]	137[a]
TGX 1955-4F	62[a]	128[a]
TGX 1485-1D	64[a]	133[a]
TGX 1987-62F	64[a]	140[a]
TGX 1945	63[a]	134[a]
SC-SAGA	55[a]	100[a]
S E ±	4.2	22.4
Interaction		
I x V	NS	NS

Means followed by the same letter within a treatment column are not significantly different at P < 0.05; Ndfa = Nitrogen derived from atmosphere; NS= Not significant

IV. CONCLUSION

In conclusion the amount of N_2 fixed and % Ndfa for the inoculation was higher than that of the uninoculated (control). This nevertheless indicates higher numbers of rhizobia can effectively increase the amount of N_2 fixed and % Ndfa. It was observed that the highest N_2 fixed (150 Kg N ha^{-1}), equivalents to 63% was by TGX 1935-3F while lowest (100 Kg N ha^{-1}) equivalent to 55% N was by SC-SAGA. It could be recommended to carry out more studies under different soil types in the same agro ecological zone.

ACKNOWLEDGMENT

The authors wish to acknowledge the support received through N2Africa project funded by The Bill & Melinda Gates Foundation.

REFERENCES

[1] R. Prasad and J. F. Power (1997.)Soil Fertility Management for Sustainable Agriculture, CRC Press, Boca Raton, Fla, USA.

[2] J. R. Bruno, R. Alves, M. Boddey and U. Segundo.(2003) The success of BNF in soybean in Brazil. Plant and Soil. Publisher: Springer Science +Business Media B.V., Formerly Kluwer Academic Publishers B.V. Issue: Volume 252, Number 1: 1–9.

[3] M.A.T. Vargas and M. Hungria. (1997) Fixaco Biologia, do N2 na culture da soja: In Biologia dosSolaos de Cerrados, ed Varga s MAT& Hungria M pp 279-360. Planatina. DF, Brazil:EMBRAPA-CPAC, ISNB 85-7075-006-4.

[4] L.S. Chen, A. Figueredo, H. Villani, J. Michajluk and M. Hungria. (2002) Diversity and symbiotic effectiveness of rhizobia isolated from field-grown soybean nodules in Paraguay. *Biology and Fertility of Soils,* 35: 448-457.

[5] D. Werner, W. E. Newton. Nitrogen fixation in agriculture, forestry, ecology, and the environment. Springer Publication. 2005

[6] Khosro Mohammadi, Yousef Sohrabi, Gholamreza Heidari; Shiva Khalesro and Mohammad Majidi.(2012) Effective factors on biological nitrogen fixation. Review: African Journal of Agricultural Research. 7(12):1782-1788.

[7] N. Sanginga, K. Dashiell, J. A. Okogun and G. Thottappilly.(1997) Nitrogen fixation and N contribution by promiscuous nodulating soybeans in the southern Guinea savanna of Nigeria. 195: (2), 257-266, DOI: 10.1023.

[8] J. M. Anderson and J.S.I. Ingram. Colorimeter determination of ammonium. In: Tropical Soil Biology and Fertility: A Hand Book of Methods. CAB. International, Wallingford, U K. 1993, 73-79.

[9] J.W. Wendt. Chemical Analysis Manual. Soil and Plant Samples. Rockefeller Foundation and Department of Agricultural Research and Technical Services, Lilongwe, Malawi. 1996, 342pp.

[10] J. M. Anderson and J. S. I. Ingram. Tropical Soil Biology and Fertility: A handbook of methods of analysis. CAB International, Wallingford, U K. 1989, 45-55.

[11] FAO (Food and Agriculture Organization of the United Nations). Fertilizer and plant nutrition bulletin. FAO, Rome, Italy, 2008, 220.

[12] S. Combs, J. Denning and K. D. Frank. Sulfate - Sulfur. pp. 35-40. In J. R. Brown (ed.) recommended chemical soil test procedures for the North Central Region. NCR Publication No. 221 (Revised). Missouri Agricultural Experiment Station SB 1001. 1998

[13] M. B. Peoples, A.W. Faizaih, B. Rerkasem and D. F. Herritedge. Methods for evaluating nitrogen fixation by nodulated legumes in the field. Canberra: ACIAR, Australia. 1989

[14] E. J. Corbin, J. Brockwell and R. R. Gault (1977). Nodulation studies on chickpea (Cicer arietinum). Australian Journal of Experimental Agriculture and Animal Husbandry (17): 126 –134.

[15] D. M. Sylvia, J. J. Furhmann, P. G Hartel and D. A. Zuberer. Principles and application of

soil microbiology. 2nd ed. Pearson Prentice Hall. New Jersey, USA. 2005

[16] F. Harry and C. A. Steven. Statistics: Concepts and Applications. Cambridge University Press, Great Britain. 1995, pp. 853.

[17] R. D. Steel and J. H. Torrie. Principles and procedures in statistics: Biometrical application. 2nd edition, MacGraw Hills Publishers, New York, USA, 1985, Pp 180.

[18] J. U. Eno, O. I. Trenchard, A. Joseph, O. Anthony, O. Abo, and E. E. Ivara. Manual of soil, plant and water analyses. 2009, Pp.51-66. ISBN 978-8012-71-X.

[19] A. O. Osunde, A. Bala, M. S Gwam, P. A. Tsado, N. Sanginga and J. A. Okogun (2003). Residual benefits of promiscuous soybean to maize (Zea mays L.) grown on farmers' fields around Minna in the southern Guinea savanna zone of Nigeria. Agriculture Ecosystems and Environment, 100: (2-3) 209-220, ISSN: 0167 - 8809.

[20] T. Solomon, M. P. Lalit and A. Tsige (2012). Effects of inoculation by Brady rhizobium strains on nodulation, nitrogen fixation, and yield of soybean (Glycine max l., merill) varieties on Nitisols of Bako, Western Ethiopia. ISRN Agronomy 12: 1-14 Article ID 261475.

[21] H.V.A. Bushby. Current perspectives in nitrogen fixation. In: Gibson, A.H. and William, E.N. (Eds.). Australian Academy of Science. 1981, 432.

[22] G. U. Okereke, C. Onochie, A. Onunkwo and E. Onyeagba (2004). Effectiveness of foreign bradyrhizobia strains in enhancing nodulation, dry matter and seed yield of soybean (Glycine max L.) cultivars in Nigeria. Biol. Fertil. Soils (33): 3-9.

[23] M. M. Tahir, M. K. Abbasi, N. Rahim, A. Khaliq and M. H. Kazmi (2009). Effect of Rhizobium inoculation and NP fertilization on growth, yield and nodulation of soybean (Glycine max L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. African Journal of Biotechnology. 8 (22): 6191-6200.

[24] W. Bekere, W. Endalkachew and K Tesfu. (2012) Growth and nodulation response of soybean (Glycine max L) to Bradyrhizobium inoculation and phosphorus levels under controlled condition in South Western Ethiopia. African Journal of Agricultural Research 7 (30):, 4266-4270.

[25] J. A. Okogun, B. T. Otuyemi and N. Sanginga. (2004) Soybean yield determinants and rhizobial inoculation in an on-farm trial in northern guinea savanna of Nigeria. Agronomy Department University of Ibadan, Nigeria. West African journal of applied ecology, 6: 344.

[26] K.O.W Njira, P.C. Nalivata, G.Y. Kanyama-Phiri and M.W. Lowole (2013). An assessment for the need of soybean inoculation with Bradyrhizobium japonicum in some sites of Kasungu district, Central Malawi. International Journal of Current Microbiology and Applied sciences. (2)8: 60-72 ISSN: 2319-7706.

[27] A. Asghar, S. Salim, H. Shaukat, I. A. Qamar and B. K. Roidar (2000). Breeding food and forage legumes for enhancement of nitrogen fixation: a review 6(1): Available at: http//www.frequently asked question.html Accessed on 15/07/2012

[28] A. O. Osunde and A. Bala (2005). Nitrogen derived from promiscuous soybean in maize-based cropping system in the moist savanna of Nigeria. Nigerian Journal of Soil Science. (15): 109-115.