Journal of Agriculture and Environment
Vol. 20 No. 1, 2024: 371-380

Suitability of indigenous rhizobia isolates as nitrogen biofertilizers for groundnut in the Nigerian Savanna

A.A. Abdullahi and G.L. Abdullahi

Department of Soil Science, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

A study was conducted in search of the best inoculant for recently released groundnut genotypes in the Nigerian Savana. The treatments were two (2) groundnut genotypes (SAMNUT 25 and SAMNUT 26) and five (5) nitrogen (N) sources; (NC 92; KBU 026; MJR 518; optimum N and uninoculated control). These were factorially combined into a total of 10 treatment combinations replicated three times in a randomized complete block design (RCBD). The plants were grown for six (6) weeks and the number of flowers, shoot dry matter and N uptake were determined. The results showed significant difference among the treatments and their interactions ($P < 0.05$), with respect to all parameters. These were used to arrive at the best inoculant in terms of N_2 fixation in symbiosis with SAMNUT 25 as KBU 026, while MJR 518 had higher capacity of N_2 fixation in symbiosis with SAMNUT 26. Even though, KBU 026 generally performed better than the latter relative to both groundnut genotypes. The study indicates variation among indigenous rhizobia isolates for suitability as potential inoculants for groundnut inoculation. It also shows the possibility of arriving at a best inoculant (KBU 026) for both genotypes.

Keywords: Groundnut; legumes; N_2 fixation; rhizobia; symbiosis

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is an important self-pollinated legume crop in tropical and subtropical regions. It is cultivated in 24 million ha world over, mainly as source of food, feed and extraction of edible oil, among other uses (Dauda *et al*., 2016). However, low yield of groundnut is usually obtained by farmers in Nigeria as result of the soils being generally low in fertility, particularly low in nitrogen and other important soil chemical properties (Machido *et al*., 2011). This makes them relatively unproductive due to continuous weathering, regular crop uptake and other soil processes (Machido *et al*., 2011). The situation necessitates the employment of inorganic fertilizers, which are costly and associated with environmental pollution at the long run (Yakubu *et al*., 2010). Therefore, the need to use technologies such as biological nitrogen fixation (BNF) between legumes and root nodule bacteria (rhizobia), that is more efficient, relatively cheaper and environment friendly. Biological nitrogen fixation has been reported as a fundamental process towards achieving sustainable agriculture because of its ability to alleviate the need to provide manufactured

Correspondence: aanchau@yahoo.com
https://dx.doi.org/10.4314/jagrenv.v20i1.34

nitrogen fertilizer (Giller *et al.*, 2016). The process has been reported to enhance groundnut production through improvement of yield and soil fertility (Asante *et al.*, 2019).

Groundnut varieties differ in their ability to improve soil fertility through biological nitrogen fixation with rhizobia strains (Jaiswal *et al.*, 2017). It has been reported that different legume genotypes respond differently to inoculation with rhizobia strains (Abdelmalik *et al*., 2015). This has been attributed to variability in genetic composition among the groundnut genotypes. These influence the quantity of nitrogen they could fix in association with particular rhizobia strains (Joint, 1998). Host specificity is an intriguing but poorly understood property of legume-rhizobia symbiosis, controlled by both the rhizobia and legume genes. Therefore, groundnut response to inoculation has been inconsistent, especially in soils with high population of indigenous rhizobia (Yusuf *et al*., 2011). Some studies have indicated increased yield, following inoculation of legumes with compatible microsymbionts (Badawi *et al.*, 2011; Yusuf *et al*., 2011), while others showed higher yields in control plots that were not inoculated as a result of higher compatibility of the indigenous rhizobia with the legumes, particularly, in fields with high population of effective indigenous rhizobia (Koskey *et al*., 2017). Hence, the need for selection of effective native rhizobia adapted to particular environments for increased productivity of the groundnut crop through biological nitrogen fixation. Employment of biotechnology, particularly rhizobiology in agriculture has been reported as inadequate (Machido *et al*., 2011). Very limited studies have been conducted, hence, the in availability of a standard inoculant for most legumes grown in Nigeria, with the exception of soya bean (Abdullahi and Yusuf, 2017). Currently, there is no recommended inoculant for groundnut in the country, while improved groundnut genotypes are continuously released for commercial production. These groundnut genotypes have not been tested for their response to rhizobia inoculation. Therefore, the need to evaluate the symbiotic efficiency of isolated indigenous rhizobia with recently released groundnut genotypes to determine a potential inoculant for groundnut crop in Nigeria. This study was therefore, conducted with the objectives testing the effectiveness of indigenous rhizobia isolates on SAMNUT 25 and SAMNUT 26 groundnut genotypes and to determine the best inoculant for each genotype as well as identify a potential suitable inoculant for groundnut inoculation in Nigeria.

MATERIALS AND METHODS

Study Location

The experiment was set up in the screenhouse of the Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, situated at N11^{o} 11.542 E 007^{o} 45.547 N, and 611 m above sea level in the Northern Guinea Savanna zone of Nigeria. The area has a long-term mean annual rainfall of 1101±16.1mm with uni-modal rainfall pattern, spanning from April to October. The mean monthly minimum and maximum temperature range from 20 and 12°C in December and to 35 and 28°C in April, respectively (Lawal, 2023). The topography is almost plain (nearly levelled) with a < 2% slope. The soil type is Typic Haplustults derived from pre-Cambrian crystalline basement complex rocks with some quaternary aeolian deposits (Lawal, 2023).

Collection and Preparation of Experimental Soil

The experimental soil was a combination of field soil and river sand. The field soil was collected at a depth of 0-20 cm from the research farm of the Institute for Agricultural

Research (IAR), Ahmadu Bello University Zaria field at N11^{o} 10.510 E 007^{o} 36.653 N and 658 m above sea level in the Northern Guinea Savanna zone of Nigeria. The field was low in nitrogen and legume was not cultivated prior to the experiment. While the river sand was collected from the stream with within the environment, which do not contain nutrients. A portion of the experimental soil was air dried, crushed and sieved through 5 mm mesh sieve and used for planting, while another portion was sieved through 2 mm mesh sieve for physical and chemical analysis using standard methods (IITA, 1982). The soil sieved through a 5 mm mesh sieve and the river sand were mixture in 1:1 ratio sterilized in the autoclave at 121^{o}C foe 15 minutes to eliminate the existence rhizobia contaminants that may interfere with the inoculants, as according to Tiwari *et al*. (2012). This mixture of both the sandy loam soil and river sand was filled into plastic pots of 16.5 cm height with a capacity of 4 kg. The pots were sterilized with 3% v/v sodium hypochlorite, while the sand mixture was autoclaved (Tiwari *et al.,* 2012). The soil in experimental pots was then leached thoroughly with boiled DI water. They were then covered with polyethene material and allowed to drain freely overnight prior to seed sowing. The bench in the screen house was adequately sterilized with 70% v/v ethanol before placing the pots. Hence, the experiment was conducted under axenic conditions.

Treatments and Experimental Design

The experiment involves two factors; 5 nitrogen sources, uninoculated (-N) control, optimum N (10 ml of 10 kg L^{-1} KNO_3 weekly (Abdullahi, 2018), NC 92 (an internationally standard commercial rhizobia strain for groundnut) obtained from the Centre for Rhizobium Studies, Murdoch University, Australia, two rhizobia strains KBU 026 and MJR 518 isolated from the Northern Guinea and Sudan savannas of Nigeria, respectively. The test crops were two groundnut genotypes; SAMNUT 25 and SAMNUT 26, among those recently released by the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. These make up ten treatment combinations, replicated three times in a Randomized Complete Block Design (RCBD) to take care of gradient due to solar radiation within the screenhouse as a result of nearby trees canopy cover.

Surface Sterilization of Seed and Sowing

The seeds were surface sterilized by immersing in 70% (v/v) ethanol for 10 seconds, followed by 3 minutes in 3% (v/v) sodium hypochlorite solution and finally rinsed in six fresh changes of sterile deionized (DI) water. They were then separately set for pre-germination in sterile plastic lunch boxes, lined with moist papers towels and then wrapped with aluminium foil. They were placed in the incubator at 28^{o}C for 3 days. Four pre-germinated seeds were then sown per pot. One millitre of the relevant inoculant of KBU 026 and MJR 518 prepared using standard procedures was used to drench each seed. The surface of the pots was then covered with gravels of average 5 mm diameter, just enough to cover the surface of the soils from contaminants deposition and act as mulch to minimize moisture loss. The plants were thinned to two per pot, at two weeks after sowing. The plants were then uniformly watered with sterile DI water. Similarly, N free CRS nutrient solution (Tiwari *et al*., 2012) was applied to all the pots. weekly While 10 millitres of 10 g L^{-1} KNO_3 only applied to positive N control weekly. Nutrient solutions and DI water were applied through sterile polyvinyl chloride tubes (25 cm in diameter) inserted into the centre of each pot.

Harvest and Data Collection

The plants were harvested at six weeks after planting and the number of flowers, shoot dry matter weight and N uptake were determined. The shoots were oven dried at 65°C for 48 hours to a constant weight prior to the weighing. The shoots were ground in a stainless-steel mill and total nitrogen determined using Kjeldahl digestion method (Jacson, 1962). While N uptake was calculated as the product of shoot dry matter (g) and N concentration (%).

$$\text{N uptake in plant} = \frac{\text{dry matter weight kg/ha}}{100} \times \text{conc. of N (\%) in plant} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

(Unkovich *et al*., 2008)

Statistical Analyses

Analysis of variance was employed using IBM Statistics 20 to determine significant differences among the treatments. Where significant difference exists, Duncan multiple range test (DMRT) was used to separate the means.

RESULTS

Physical and Chemical Properties of the Experimental the Experimental Soil

Results of some physical, chemical and microbiological properties of the experimental soil is shown in Table 1. The particle size distribution result shows that the experimental soil is loamy sand, soil reaction (pH) of the soils in both water and $CaCl_2$ were strongly acidic; 5.45 and 4.61 respectively, using the rating of FMANR (1990). Organic carbon was very low (0.80 g kg^{-1}), total N of the unleached soil was 0.56 g kg^{-1} while that of the leached soil was 0.45, both very low, available P was moderate with value of 11.83 mg kg^{-1}, while the cation exchange capacity (CEC) was medium (11.1 cmol kg^{-1}). The microbial biomass carbon was also low indicating low population of microbes, 54.68 mg kg^{-1}.

Table 1. Physical, chemical and microbiological analysis of soil

Parameter	Amount
pH	5.45
Organic carbon (g kg^{-1})	8.00
Total nitrogen unleached (g kg^{-1})	0.50
Total nitrogen of leached (g kg^{-1})	0.45
Available phosphorus (mg kg^{-1})	11.83
CEC (cmol kg^{-1})	11.10
Textural Class	Loamy sand
Microbial biomass C (mg kg^{-1})	54.68

Effect of Inoculation and Groundnut Genotypes on Number of Flowers

The response of the number of flowers of SAMNUT 25 and SAMNUT 26 to the two rhizobia isolates is shown in Figure 1. There was significant difference ($P < 0.01$) among the treatments in influencing the number of flowers of the groundnut genotypes. The highest

number of flowers was obtained from inoculation of SAMNUT 25 with KBU 026, which was significantly higher than inoculation of both SAMNUT 25 and SAMNUT 26 with NC 92, inoculation of SAMNUT 26 with MJR 518 as well as application of optimum N to both SAMNUT 25 and SAMNUT 26 and leaving SAMNUT 25 uninoculated, which were statistically similar and higher than the rest of the treatments (inoculation of SAMNUT 25 with NC 92 and MJR 518, inoculation of KBU 026 with KBU 026 as well as leaving SAMNUT 26 uninoculated). However, inoculation of SAMNUT 25 with NC 92 and MJR 518 were statistically similar and higher than inoculation of SAMNUT 26 with KBU 026 and leaving SAMNUT 26 uninoculated. Likewise, inoculation of SAMNUT 26 with KBU 026 was statistically higher than leaving SAMNUT 26 uninoculated, which gave the lowest number of flowers. Inoculation of SAMNUT 25 with KBU 026 resulted in 50% higher number of flowers relative to the uninoculated control. On the other hand, inoculation of SAMNUT 26 with MJR 518, NC 92 and application of optimum N resulted in 79, 66 and 75% higher number of flowers, respectively relative to the uninoculated controls.

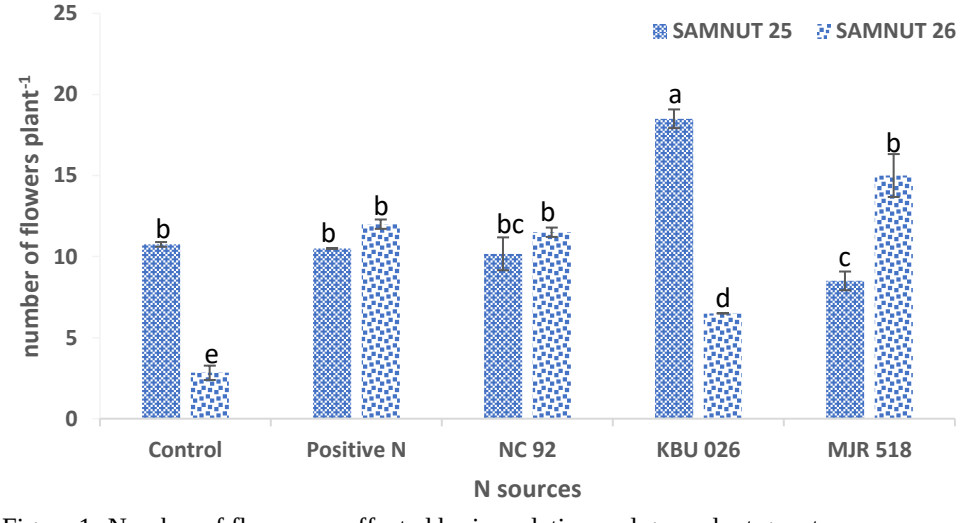

Figure 1**:** Number of flowers as affected by inoculation and groundnut genotypes

Effect of Inoculation and Groundnut Genotypes on Shoot Dry Matter

Figure 2 shows the response of two groundnut genotypes to nitrogen sources in terms of shoot dry matter. There was significant difference ($P < 0.01$) difference among the treatments in influencing the shoot dry matter of the groundnut genotypes. The highest shoot dry matter was observed when SAMNUT 26 was inoculated with MJR 518, which was statistically similar to inoculation of SAMNUT 25 with KBU 026 and SAMNUT 25 was applied with the optimum N. Even though, the application of optimum N to SAMNUT 25, inoculation of SAMNUT 26 with KBU 026 and NC 92 and application of optimum N to SAMNUT 26inoculation of SAMNUT 26 with MJR 518 and leaving SAMNUT 25 uninoculated were also statistically similar and higher than the rest of the treatments. Likewise, the application of optimum N SAMNUT 26, inoculation of both groundnut genotypes with NC 92, inoculation of SAMNUT 25 with MJR 518 and leaving SAMNUT

25 uninoculated were also statistically similar and higher than the rest of the treatments. Similarly, leaving SAMNUT 25 uninoculated, inoculation of both groundnut genotypes with NC 92, inoculation of SAMNUT 25 with MJR 518, inoculation of SAMNUT 26 with KBU 026 and application of optimum N to KBU 026 were also statistically similar and higher than leaving SAMNUT 26 uninoculated and inoculation of SAMNUT 26 with KBU 026, which in turn were also statistically similar. However, inoculation of SAMNUT 26 with KBU 026 was statistically higher than leaving SAMNUT 26 uninoculated, which gave the lowest shoot dry matter. Inoculation of SAMNUT 25 with KBU 026, application of optimum N and inoculation with NC 92 superseded the uninoculated control by 40, 13 and 7% in shoot dry matter development of the same genotype.

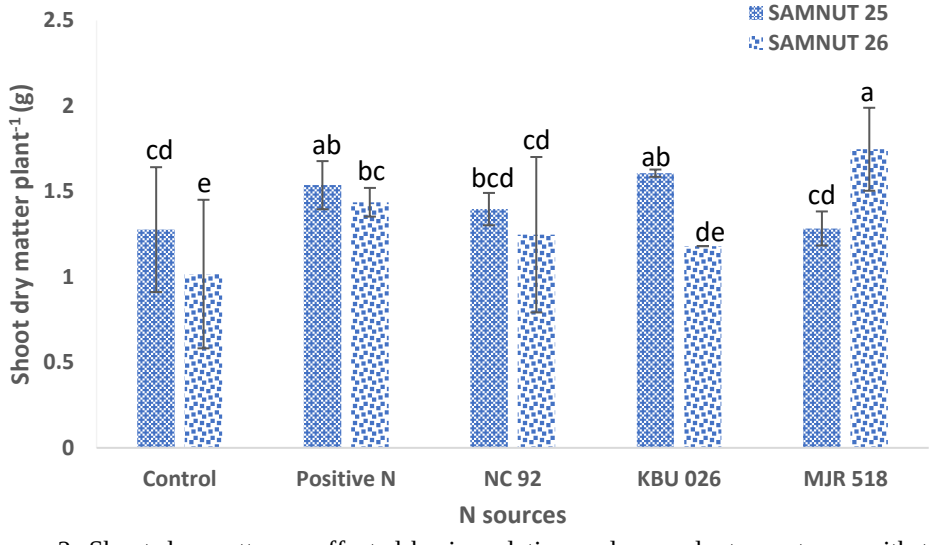

Figure 2: Shoot dry matter as affected by inoculation and groundnut genotypes with two indigenous rhizobia isolates.

Effect of Inoculation and Groundnut Genotypes on N Uptake

The effect of the N sources on the N uptake of the two groundnut genotypes is shown in Figure 3. The highest N uptake obtained from the application of the optimum N to SAMNUT 25, which was statistically higher in influencing N uptake than inoculation of SAMNUT 25 with KBU 026 and inoculation of SAMNUT 26 with MJR 518, which were statistically similar and higher than the rest of the treatments. In turn, the application of optimum N to SAMNUT 26, inoculation of both groundnut genotypes with NC 92 and inoculation SAMNUT 26 with KBU 026 were also statistically similar and higher than the other treatments. Likewise, application of optimum N to SAMNUT 26, inoculation of SAMNUT 26 with NC 92 and inoculation of SAMNUT 25 with MJR 518 and higher than the rest of the treatments. Even though, inoculation of SAMNUT 25 with MJR 518 and leaving both groundnut genotypes uninoculated were also statistically similar. However, leaving both groundnut genotypes uninoculated gave the lowest N uptake. There was 67, 40, 34 and 32% higher N uptake of SAMNUT 26 as a result of inoculation with MJR 518, inoculation with KBU 026, inoculation with NC 92 and application of optimum N,

respectively. Similarly, application of optimum N, inoculation with KBU 026, inoculation with NC 92 and inoculation with MJR 518 gave 70, 68, 50 and 13% higher N uptake compared to the inoculated control for the same groundnut genotype.

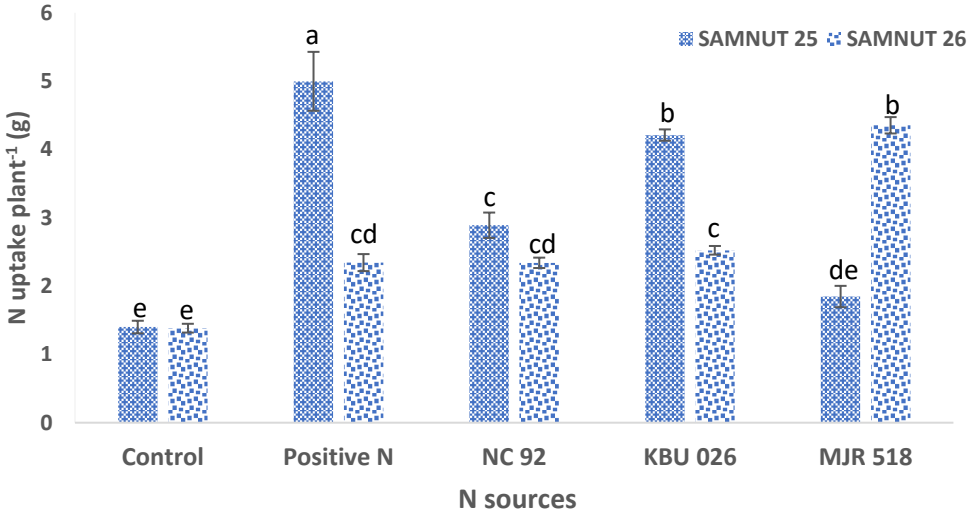

Figure 3**:** N uptake as affected by inoculation and groundnut genotypes

DISCUSSION

Results of soil analysis show that the soil is low in fertility status according to the rating of Esu (1991). Savanna soils have been largely reported as low in CEC and organic carbon, which has been attributed to rapid mineralization of organic materials due to high temperature (Sharu *et al.,* 2013). The low available P could be linked to low P content of organic residues, which is the main source of P to the soil that is very little in quantity. Phosphorus also depleted through crop removal along with the other nutrients or fixed into insoluble forms due acidic soil conditions as shown by the soil analyses results (Collavino *et al*., 2010). The low N content of the soils may also be attributed to the earlier pointed low organic matter content of the soil, since inorganic N, usually in form of nitrates and nitrites accounts for only a small portion of total N for savanna soils (Sharu *et al.*, 2013). This was further decreased through leaching of possible nitrates in the experimental soil with hot water to avoid interference of available nitrogen in the experiment, which is known to reduce nodulation and the rate of N_2 fixation. The microbial biomass of carbon is very low as shown in Table 1, confirming the low organic matter content of the soil and indicating low microbial activity, probably caused by the acid condition that limits the survival of most microorganisms.

The observed response of the yield parameters to N_2 fixation induced by the indigenous rhizobia being similar to the effect of application of optimum N and the standard reference strain (NC 92), indicates that both inoculants could have supplied similar amount of N relative to the requirement of the crop (Sanginga *et al.,* 2000). Mathenge *et al*. (2016) reported that the potential of legumes to fix dinitrogen to be high when the mineral N of the

soil is low compared to conditions of soils with richer mineral N. Abdelmalik *et al.* (2015), also reported variation in response of legume species or cultivars under different environmental and soil conditions, to inoculation with different rhizobia strains. Flavonoids and other factors released by the groundnut genotypes to the soil environment may differ in the rhizobia genotype they attract and form nodules with through their Nod factors (Roy *et al*., 2020). Hence, variation in the degree of symbiosis and the amount of N that could be fixed from the atmosphere by the relationship (Tiwari *et al*., 2012). Hence, SAMNUT 25 could have been more compatible with KBU 026, while SAMNUT 26 more compatible with MJR 518. Generally, KBU 026 has higher tendency to perform better in conversion of atmospheric nitrogen from the atmosphere for utilization by both groundnut genotypes and possibly other ones to be bred as earlier observed by Abdullahi (2018) on SAMNUT 22 and SAMNUT 24 groundnut genotypes.

CONCLUSION

The two indigenous rhizobia isolates (KBU 026 and MJR 518) proved their compatibility with the two groundnut genotypes, competing favourably with the optimum N application and performing higher than the reference strain NC 92. However, NC 92 was superior when compared to uninoculated control. The study revealed higher performance of SAMNUT 25 when inoculated with KBU 026, while SAMNUT 26 performed higher when inoculated with MJR 518, even though, KBU 026 generally performed higher than the MJR 518 irrespective of the two groundnut genotypes. This indicates variation among indigenous rhizobia for suitability as potential inoculants for groundnut genotypes. KBU 026 could be considered as a more suitable inoculant for different groundnut genotypes.

REFERENCES

Abdelmalik, O.A., Idris, Awad, Abdel Rahim M. and Abdelmonem S.E. (2015). Response of groundnut to nitrate and inoculation with bacteria isolated from three different regions in Sudan and its effect on mineral content of the three different regions in Sudan and its effect on mineral content of the shoot system. *International Journal of Scientific Research in Knowledge*, 3(10), 0247-254.

Abdullahi, A.A. (2018). Exploring the genetic diversity of groundnut (A*rachis hypogaea* l.)-nodulating rhizobia in Northern guinea and Sudan savannas of Nigeria for increased nitrogen fixation and productivity. PhD thesis. Department of Soil Science, Ahmadu Bello University, Zaria, Nigeria. 294pp

Abdullahi, A.A. and Yusuf, A.A. (2017). Higher effectiveness of extant indigenous groundnut (Arachis hypogaea L.-nodulating rhizobia over introduced inoculant strains on an Alfisol. in sustainable soil management and food and nutrition security. Proceedings of the 41st Annual Conference of the Soil Science Society of Nigeria, Abubakar Tafawa Balewa University, Bauchi, Nigeria. 13th-17th March, 2017. pp. 164-172.

Badawi, F.S.F., Biomy, A.M.M. and Desoky, A.H. (2011). Peanut plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizo-microorganisms under sandy loam soil conditions. *Annals of Agricultural Science*, (56): 17-25.

Collavino, M.M., Sansberro, P.A., Mroginski, L.A., and Aguilar, O.M. (2010). Comparison of vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid

soil and their ability to promote *Phaseolus vulgaris* growth. *Biology and Fertility of Soils*, 46:727-738.

Dauda, H., Mukhtar A.A., Babaji, B.A. and Olufajo, O.O. (2016). Yield response of groundnut (*Arachis hypogaea*), varieties to gypsum and rhizobia inoculation in the Nigerian savanna. *Gashua Journal of Irrigation and Desertification Studies*, 12: 14-15

Esu, I.E. (1991). Detailed soil survey of NIHORT farm at Bunkure Kano state, Nigeria. Institute for Agricultural Research Samaru, Zaria, Nigeria.

FMANR (1990). Literature Review on Soil Fertility Investigations in Nigeria (in five volumes). Federal ministry of Agriculture and Natural Resources, Abuja, p 281.

Giller, K.E., Herridge, D.F. and Sprent, J.J. (2016). Legume-rhizobia symbiosis and assessing the need to inoculate. *In*: J. G. Howieson and M. J. Dilworth (Eds.). *Working with Rhizobia*. 25-37 Australian Centre for International Agricultural Research (ACIAR) Canberra, Australia.

IITA (1982). International Institute of Tropical Agriculture. Automated and semi-automated methods of soil and plant analysis. *IITA manual series* 7:33.

Jaiswal, S.K., Msimbira, L.A., and Dakora, F.D. (2017). Phylogenetically diverse group of native bacterial symbionts isolated from root nodules of groundnut group of native bacterial symbionts isolated from root nodules of groundnut (*Arachis hypogaea* L.) in South Africa. *Systematic and Applied Microbiology*, 40, 215–226.

Joint, F.A.O. (1998). Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia. Results of a coordinated research programme (No. IAEA-TECDOC--1027). Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Koskey, G., Mburu, S.M., Njeru, E.M., Kimiti, J.M., Ombori, O. and Maingi, J.M. (2017). Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (Phaseolus vulgaris L.) in contrasting environments of Eastern Kenya. *Frontiers in Plant Science,* 8 article 443, 1-12.

Lawal, H.M. (2023). Sequestration of soil organic carbon pools under diverse tillage practices and cover crops in Northern Guinea Savanna, Nigeria. *Nigerian Journal of Soil and Environmental Research*, 22:29-46.

Machido, D.A., Olufajo, O.O., Yakubu, S.E. and Yusufu, S.S. (2011). Enhancing the contribution of the legumes to the N fertility of soils of the semi-arid zone of Nigeria. *African Journal of Biotechnology*, 10 (10), 1848-1853.

Mathenge, C., Thuita M., Gweyi-Onyango J. P. and Cargele, M. (2016). Soybean (Glycine max) response to rhizobia inoculation and soil nitrogen. Department of Agricultural Science and Technology, Kenyatta University, and International Institute of Tropical Agriculture, Nairobi, Kenya, 35: 45-38

Roy, S., Liu W., Nandety R. S., Crook A., Mysore K. S., Pislariu C. I., Frugoli J., Distein, R. and Udvardi M. K. (2020). Celebrating 20 years of genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation. *Plant Cell*, 32:15-41.

Sanginga, P.C., Adesina, A.A., Manyong, V.M., Otite, O. and Dashiell, K.E. (1999). Social impact of soybean in Nigeria‟s southern Guinea savanna. International Institute of Tropical Agriculture, 4-6.

Sharu, M., Yakubu, M. and A. I. Tsafe, S.N.A. (2013). Characterization and classification of soils on an agricultural landscape in Dingyadi District, Sokoto State, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 21(2): 137-147.

Tiwari, R., Ardley, J., Carr, R., Parkinson, L. and Marfisi, A. V. (2012). Master class in rhizobial technology; 'Isolation, identification and utilisation of root nodule bacteria (rhizobia) in promoting sustainable agricultural productivity'. 2nd-13th December, 2012, Sri Lanka.

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, B. Giller, K. and Chalk, P. (2008). Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph No. 136, 258 pp.

Weilbo, J. (2012). Rhizobial communities in symbiosis with legumes: Genetic diversity, competition and interactions with host plants. Central European Journal of Biology, 7(3):363-372.

Yakubu, H., Kwari, J.D. and Ngala, A.L. (2010). N_2 fixation by grain legume varieties as affected by rhizobia inoculation in the sandy loam soil of Sudano-Sahelian zone of northeastern Nigeria. *Nigerian Journal of Basic and Applied Science*, 18(2), 229-236.

Yang, S., Tang, F., Gao, M., Krishnan, H. B. and Zhu, H. (2010). R gene-controlled host specificity in the legume–rhizobia symbiosis. *Proceedings of the National Academy of Sciences of United states of America*, 107(43), 18735-18740.