



Phosphorus accumulation in soybean (*Glycine max* (L.) Merrill) varieties inoculated with indigenous Rhizobial isolates in soils with low available phosphorus

¹Ojo Abisoye O., ²Dare, M. O., ¹Olubiyi, M. R. and ¹Olosunde*, A. A.

¹National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation Ibadan, Nigeria.

²Tiani Gardens, 608 West Street, Stoughton MA 02072 USA.

Abstract

Phosphorus (P) is one of the important plant macronutrients in the soil. Low soil P affects rhizobia-legume symbiosis. P content and uptake of three soybean varieties (TGx1448-2E, TGx1908-1F and TGx1910-2F) inoculated with four indigenous rhizobial isolates (TRC2, IDC8, OISa-6e, R25B+ IRj1280A) and control was studied in three locations (the University of Ibadan Teaching and Research Farm (UITRF), Idi-Ayunre (IA) and Orile-Ilugun (OI)) in a randomized complete block design. The available P of the three locations ranged between 0.06 – 0.42 (mg/kg) and no P fertilizer was applied in the experiment. Rhizobial inoculation had no significant ($P < 0.05$) effect on the shoot and P content (% dry matter), shoot P uptake (kg/ha) and arbuscular mycorrhizal colonization (%) of the soybean. However, grain P uptake (kg/ha) was significantly ($P < 0.05$) higher in OISa-6e and R25B+ IRj1280A inoculated plants compared to other inoculation treatments (TRC2 and IDC8). There was about a 25% increase in the grain P uptake of the two isolates (OISa-6e and R25B+ IRj1280A) when compared to the control. The study revealed the minimal effect of rhizobial inoculation on P accumulation in soybean under low P soils of the studied area.

Keywords: Soybean; Rhizobial inoculation; arbuscular mycorrhizal; colonization.

E-mail Address: : olosundam@yahoo.com (+234) 805 916 7889

<https://doi.org/10.36265/colssn.2021.4551>

©2020 Publishingrealttime Ltd. All rights reserved.

Peer-review under responsibility of 45th SSSN Conference LoC2021.

1.0 Introduction

Phosphorus is one of the major plant nutrients in the soil. Some of the key functions of P in the plant include energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the other. It is an essential ingredient for rhizobium to convert atmospheric atmosphere to N_2 to NH_3 . It constitutes about 0.2 % of plant dry weight (Schactman *et al.*, 1998). However, P is a major limiting nutrient for plant growth, the least mobile and least available to plants in the soil (Ahmed *et al.*, 1996) due to reactivations of phosphate ions with numerous soil constituents such as clay lattices which result in strong retention of P in most soils and thus limit plant P uptake. Vesicular arbuscular mycorrhizal is known to be helpful in the absorption of P in the soil.

Soybean (*Glycine max* (L.) Merrill) is an important grain legume and a major source of dietary protein and oil in Nigeria (Nwosu *et al* 2019). The crop was introduced into the country in the early 70s and it has become one of the important staple foods in the country. It is well established that soybean fix N thereby reducing the cost of fertilizer in the smallholder agricultural setting in Nigeria.

P has been identified as one of the most important nutrients for legume production. Research has proved that yield of soybean is greatly reduced if the soil available P is below the threshold level of 10-15 mg/kg (Vanlauwe 2019) indicating that P is an essential nutrient required for the production of soybean. Inoculation of soybean is important for N fixation and grain yield. However, for N fixation a lot of P is required. Biological nitrogen fixation is dependent on the

P source and rate of P applied. The research process in legumes has shown that P enhances the symbiotic N fixation process in legumes, hence P is an important nutrient required for most legumes. Chien *et al.* (1993) reported the effect of phosphate rock source on soybean BNF and showed that all the P treatments applied significantly increased BNF in soybean. Watkin *et al.* (1997) reported that the amount of P needed by some rhizobial strains is less than 0.5 μ mole P and where the concentration is high in the external environment the growth can be affected. Contrary to this, Ibibijen *et al.* (1996) and Vazquez *et al.* (2002) reported that rhizobium symbiosis depends greatly on high P concentration, hence an increase in plant phosphorus uptake as a result of AM colonization can lead to an increase in nodulation and the rate at which N is fixed.

Role of P in Symbiotic N fixation include increase top and root growth, decrease the time needed for the development of nodules to become active and benefit to the host legume, increase the number and size of nodules and the amount of N assimilated, per weight of nodule, increasing the percentage of total N accumulated in the harvested portion of the host plant. Improving of the density of rhizobia bacteria in the soil surrounding the root. Inoculant has always been advised even for the promiscuous soybean varieties (Abaidoo *et al.*, 2007; Giller *et al.*, 2011). Brockwell *et al.* (1995) expatiated further, by reporting that the introduced rhizobial strain must be able to compete, establish and persist with other microflora to form effective nodules in the introduced environment. This study, therefore, investigated the effect of inoculation on the P concentration, P uptake (shoot and grain), grain yield, residual soil P and vsm colonization.

2.0 Materials and Methods

Location, climates and soils of the experimental sites
Multilocal trial was conducted in three different locations namely: Idi-Ayunre (IA), Orile-Ilugun (OI) and the University of Ibadan Teaching and Research Farm (UITRF) within the rainforest-savanna transition zone of Nigeria. The coordinates and soil characteristics of the location are as described in Table 1. The rhizobial population count of the three locations was determined using the Most Probable Number (MPN) (Somasegaran and Hoben, 1994). Two soybean varieties, TGx1448-2E and TGx 1456-2E, and one cowpea variety IT89KD-288 seeds were sterilized, pre-germinated and transplanted into sterilized growth pouches containing modified Jensen's N-free nutrient solution (Roughley, 1984). A 5-fold dilution series (5^{-1} – 5^{-6}) with four replicates was used to inoculate each plant in the growth pouch one week after planting.

Rhizobial Isolates

Rhizobial strains IDC8, OISa-6e and TRC2 were highly infective indigenous rhizobia isolated from the soils of locations (Ojo *et al.*, 2014). Rhizobial strain R25B and IRj2180A, used as reference strains, were collected from IITA and termed exotic in this study. Rhizobial strain R25B and IRj2180A were isolated in Zaria (Sanginga 1996)

Soybean varieties

The three soybean varieties were promiscuous types. Varieties TGx 1448 – 2E and TGx 1910 – 2F are late maturing varieties that mature at about 105 – 120 Days while TGx 1908 – 1F are early maturing ones that mature in less than 95 DAP. Records have shown that TGx 1448 – 2E and TGx 1456 – 2E are very efficient in nitrogen fixation (Sanginga *et al.* 2000).

Table 1. Geographical and soil characteristics of the study sites

Characteristics	Idi Ayunre	Orile Ilugun	UITRF
Latitude and Longitude	7°26'N and 3°54'E	7°13'N and 3°31'E	7°30'N and 3°45'E
Soil Classification ¹	Nitrosol	Luvisol	Alfisol
Soil Series ²	Olorunda	Apomu	Egbeda
pH (KCl)	6.55	6.09	5.76
Total N (g/kg) ³	0.23	0.17	0.08
Available P (mg/kg) ⁴	0.42	0.06	0.13
CEC (cmol/kg) ⁵	11.73	10.42	7.77
Sand (g/kg) ⁵	645	675	812.5
Clay (g/kg) ⁵	185	185	100
Silt (g/kg) ⁵	170	140	87.5
Rhizobial count (cell g-1 soil) ⁶	3.8	7.81	13.54

¹ USDA 2006 ² Smyth and Montgomery 1962 ³ Bremner 1982 ⁴ Bray and Kurtz 1945 ⁵ Okalebo 1993 ⁶ Somasegaran and Hoben, 1994

Table 2. Effect of rhizobial inoculation on the shoot and grain P accumulation across the three locations in the study (Main effect of Rhizobial Strains inoculation)

Strain	% Dry matter of P in Shoot/ P concentra-	Shoot P uptake(Kg/ Ha)	% Dry matter of P in Grain /P conc in Grain	Grain P uptake(Kg/Ha)
OISa-6e,	0.27	9.24	0.61	11.19 b
IDC8	0.25	7.18	0.60	10.53 b
Control (0)	0.26	7.06	0.60	8.29 c
R25B+IRJ21				
80A	0.25	8.11	0.56	13.67 a
TRC2	0.26	6.58	0.60	9.51 bc
LSD	ns	ns	Ns	***

Table 3: Effect of rhizobial inoculation on the arbuscular mycorrhizal (AM) colonization and total P uptake in two soybean varieties across the three locations in the study

train	AM	Total P uptake
OISa-6e,	71.44	20.43ab
IDC8	65.33	17.72bc
Control (0)	64.41	15.34c
R25B+IRj2180A	67.52	21.78a
TRC2	65.06	16.09c
LSD	ns	***

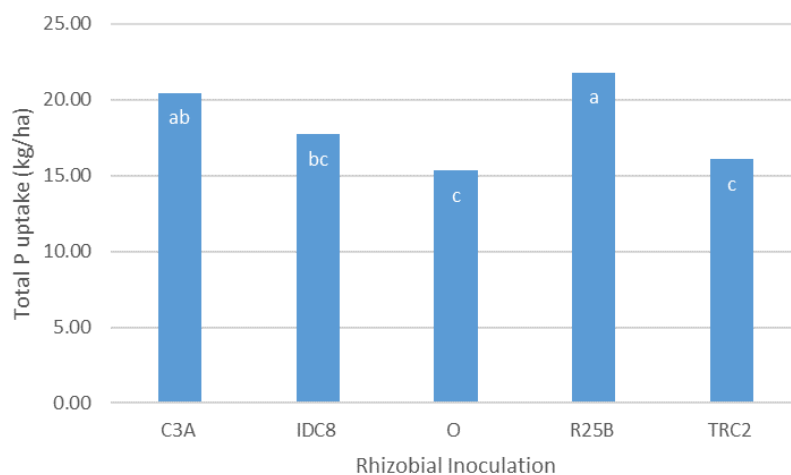


Fig 1. Total P uptake of soybean varieties in response to rhizobial inoculation across three locations

Tables 4: Shoot and grain P uptake of the soybean varieties in response to rhizobial inoculation in three locations (IA, OI and UITRF).

Rhizobial Inoculation	Grain yield (kg/ha)				Shoot dry weight (kg/ha)			
	Location				Location			
	IA	OI	UITRF	Mean ^b	IA	OI	UITRF	Mean ^b
OISa-6e,	2.21	1.25	1.94	1.80	4.38	2.23	3.21	3.27
IDC8	2.04	1.31	1.86	1.74	2.97	1.97	3.29	2.75
Control (O)	1.30	1.36	1.53	1.40	3.30	2.03	2.84	2.72
R25B+IRj218								
0A	3.58	1.36	2.21	2.38	4.92	1.88	2.64	3.15
TRC2	1.60	1.27	1.90	1.59	2.51	1.93	2.74	2.39
Mean	2.15	1.31	1.89		3.62	2.01	2.95	
SE ^a	0.19				0.45			

^aMultiply SE by 3 to compare the interactions. ^bThe mean values are the values for main effects and can be separated by LSD or alphabets.

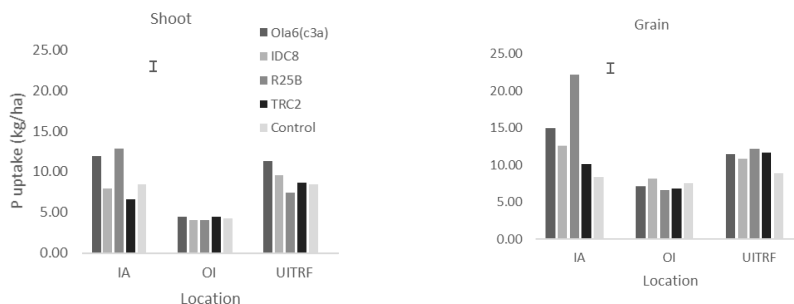


Fig 2: Shoot and grain P uptake of the soybean varieties in response to rhizobial inoculation in three locations (IA, OI and UITRF). Bars represent standard error bar

3.0 Results and Discussion

3.1 Analysis of variance

The analysis of variance revealed that rhizobial inoculation had a significant effect on the yield and P accumulation of the three soybean varieties across the three locations (Table 2). There was no significant difference in shoot % P Dry matter (P concentration in the shoot), Shoot P uptake (Kg/Ha) and % P concentration in Grain, however, P uptake in grain (Kg/Ha) was significant ($P < 0.05$) across the locations, despite the available low P across the locations R25B + IRj, inoculated soybean varieties was significantly higher in P uptake in grain than other soybean varieties inoculated with other rhizobial strains. Soybean inoculated with Isolates TRC2, IDC8 and OISa-6e were not significantly different in P uptake in grain. (Table 2). Rhizobial inoculation has no significant effect on arbuscular mycorrhizal colonization, even though the highest % colonization was obtained in soybean varieties inoculated with OISa-6e. (Table 3). There was a significant difference ($P < 0.05$) in the total P uptake. The total P uptake of soybean varieties inoculated with R25B+IRJ was not significantly different from that of OISa-6e but they were both significantly ($P < 0.05$) higher than that of IDC8 and the control (Table 3). In other words, OISa-6e and R25B+IRJ were about 25% higher in total P uptake than that of the IDC8 and the control. In Fig 1, the total P uptake of R25B+IRJ was significantly ($P < 0.05$) higher than that of the IDC8 and that of the control across the locations. The interactive effect shows that the total P uptake in shoot and grain of inoculated soybean varieties in AI and UIT&RF were both significantly ($P < 0.05$) higher than that of OI.

Soybean response to rhizobial inoculation is influenced by soil chemical and biological properties which include pH, N and P availability and indigenous rhizobial population (Thies *et al.*, 1992; Giller 2001; Osunde *et al.*, 2003; Ronner *et al.*, 2016). The rhizobial count of the three locations in our study was generally low with the highest of 13 cells per gram soil found at UITRF. Population of indigenous rhizobia in most tropical soils are low (Ahmad, 1981; Ahmad and Mchaughing, 1985, Sanginga, 2000a) and response of crops to inoculation is likely to occur when the rhizobial count is less than 10 cells per gram of soil (Thies *et al.*, 1991, 1992; Sanginga *et al.*, 1996; Okogun and Sanginga, 2003). In our study, soybean response to inoculation varied across locations. Even with low available P in all the study sites, IA had the highest content compared to the other study sites. The importance of adequate P for increased legume yield is well documented (Giller and Cadisch, 1995; Didagbé *et al.*, 2014; Ronner *et al.*, 2016). According to Vanlauwe *et al.* (2019), the overall mean effects of inoculation and P on grain yields of soybean when applied alone were often in the region of an extra 0.5t ha⁻¹ of yield. Higher soil pH also improves soil bacterial activities (Sanginga and Woome, 2010) which can lead to higher soil organic matter, mineralization and nutrient cycling that are ingredients for improved plant growth and yield.

Compatibility of strains with legume varieties and genotypes is an important factor that can affect the effectiveness of legume symbioses (Bulland *et al.*, 2005). It is important to note the effectiveness of inoculation of R25B+IRj2180A. This exotic strain had outstanding adaptation across the locations with their influence on P uptake. Vanlauwe *et al.* (2019) reported that most exotic strains used in inoculants have broad adaptability with outstanding performance in a broad range of soil. The outstanding performance of R25B+IRj2180A may also be due to the superiority of the combination of strains R25B and IRj2180A, an inoculant factor that has been report-

ed to increase yield in legumes (Chibeba *et al.*, 2018; Campo *et al.*, 2009). The inoculation of the two isolates showed responses that were comparable to that of the exotic strains R25B+IRj2180A, especially at UITRF. It is imperative to screen the rhizobial population for compatibility and effectiveness in the selection process of locally adapted strains for inoculant production for legumes. There are reports on the compatibility and effectiveness of some indigenous strains for the improvement of biological N fixation in promiscuous soybean cultivars in sub-Saharan Africa (Abaidoo *et al.*, 2000, 2007; Klogo *et al.*, 2015; Gyogluu *et al.*, 2016).

Legumes including soybean often depend on soil N at the expense of N fixation when the soil N is at a high or moderate level (Herridge and Rose, 2000; Beyan *et al.*, 2018). The average % NDFA was generally low at IA (<35%), a location with a moderate level of soil N, compared to the other two locations (OI, 45% and UITRF, 57%) with the low level of soil N. Bender *et al.* (2015) also mentioned that the soil contributes a large portion of N accumulated in soybean despite their to fix N and this probably explains higher biomass N uptake (grain and shoot) at IA. The inoculation of all the soybean varieties with R25B+IRj2180A and TGx1448-2E with OIa6(c3a) and IDC8 improved N uptake at IA. The total N accumulations in the grain and shoot of the three soybean varieties in the three locations were within the range reported by Flannery (1986). The high harvest index of N in the three locations corroborated previous studies on soybean (Bender *et al.*, 2015; Barth *et al.* 2018). This high accumulation in the grain represents a large removal of N from the soil and therefore inoculation of seed with effective inoculants is important for the sustainability of soybean production even with fertilizer input.

4.0 Conclusion

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

Acknowledgement

The International Institute of Tropical Agriculture Ibadan Nigeria provided the facilities and technical support for the study. The authors acknowledge the contribution of Late Dr. J.A. Okogun whom we started this research together but has passed away. Also, the technical input of Mr. Isaac Oloyede is appreciated.

References

- Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Borthakur, D., 2000. Bradyrhizobium spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. *Int. J. Syst. Evol. Microbiol.* 50, 225–234.
- Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Dashiell, K.E., Sanginga, N., 2007. Population size, distribution,

- and symbiotic characteristics of indigenous Bradyrhizobium spp. that nodulate TGx soybean genotypes in Africa. *Appl. Soil. Ecol.* 35, 57–67. <http://dx.doi.org/10.1016/j.apsoil.2006.05.006>. (ISSN: 09291393)
- Ahmad, M. H., McLaughling, W., 1985. Ecology and genetics of tropical Rhizobium species. *Biotechnology Advance.* 3: 155 – 170.
- Ahmad, N., 1981. Growth of *Leucaena leucocephala* in relation to soil pH, nutrient levels and rhizobium concentration. *Leucaena Research Report* 2: 5 – 10.
- Ahmad, N., Rashid, M. and Vaes, A. G. 1996. Fertilizer and their uses in Pakistan (Nutritional Fertilizer Development Center Government of Pakistan): In Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilising bacteria and a mixed rhizobial culture. *Australian Journal of Experimental Agriculture* 44: 623-628
- Barth, G., Francisco, E., Suyama, J. T., Garcia, F., 2018. Nutrient Uptake Illustrated for Modern, High-Yielding Soybean. *Better Crops/Vol.* 102: 11-14
- Bender, R.R., Haegerle, J.W., Below F.E., 2015. Nutrient uptake, partitioning, and remobilization in modern soybean varieties. *Agron. J.* 107:563–573. doi:10.2134/agronj14.0435
- Beyan, S.M., Wolde-meskel, E., Dakora, F.D., 2018. An assessment of plant growth and N₂ fixation in soybean genotypes grown in uninoculated soils collected from different locations in Ethiopia. *Symbiosis* 75: 189-203. <https://doi.org/10.1007/s13199-018-0540-9>
- Bray, R. H., Kurtz, L. T., 1945. Determination of total organic and available form of phosphorus in soils. *Soil Sci. J.* 59: 39-45.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen -total. In: Page AL, Miller, RH, Keeney DR (eds), *Methods of soil analysis, part 2.* Amer. Soc. Agron. 9:595–624.
- Brockwell, J., Botteonley, P. J. and Thies, J. E. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant and Soil* 174: 143 – 150.
- Bulland, A.O., Roughley, R. J. and Pulsford, D. J. 2005. The legume inoculant industry and inoculant quality control in Australia: 1953 – 2003. *Aust. J. Exp. Agric.* 45 (2-3): 127 – 140.
- Campo, R.J., Araujo, R.S., Hungria, M., 2009. Nitrogen fixation with the soybean crop in Brazil: compatibility between seed treatment with fungicides and bradyrhizobial inoculants. *Symbiosis* 48, 154–163. <http://dx.doi.org/10.1007/BF03179994>. (ISSN: 1878-7665).
- Chibeba, A.M., Kyei-Boahen, S., Guimarães, M.F., Nogueira, M.A., Hungria, M., 2018. Feasibility of transference of inoculation-related technologies: a case study of evaluation of soybean rhizobial strains under the agroclimatic conditions of Brazil and Mozambique. *Agric. Ecosyst. Environ.* 261, 230–240. <https://doi.org/10.1016/j.agee.2019.106583>
- Chien, S. H., Carmona, G., Menon, R. G. and Hewums, D.T. 1993. Effect of phosphate rock source on BNF by soybean. *Fertilizer Research* 34: 153 – 159.
- Didagbé, O.Y., Hounngandan, P., Sina, H., Zoundji, C.C., Kouelo, F.A., Lakou, J., Toukourou, F., Moussa, L.B., 2014. Response of groundnut (*Arachis hypogaea* L.) to exogenous Bradyrhizobium sp strains inoculation and phosphorus supply in two agroecological zones of Benin, West Africa. *J. Exp. Biol. Agric. Sci.* 2, 623–633.
- Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems, 2nd ed. CAB International, Wallingford.
- Giller, K.E., Cadisch, G., 1995. Future benefits from biological nitrogen fixation: an ecological approach to agriculture. *Plant Soil* 174, 255–277. <http://dx.doi.org/10.1007/BF00032251>.
- Giller, K.E., Murwira, M.S., Dhlwayo, D.K., Mafongoya, P.L., Mpepereki, S., 2011. Soybeans and sustainable agriculture in southern Africa. *Int. J. Agric. Sustain.* 9, 50–58. <http://dx.doi.org/10.3763/ijas.2010.0548>. (ISSN: 1747-762X).
- Giovanetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots *New Phytology* 84:489-500.
- Gyogluu, C., Boahen, S.K., Dakora, F.D., 2016. Response of promiscuous-nodulating soybean (*Glycine max* L. Merr.) genotypes to Bradyrhizobium inoculation at three field sites in Mozambique. *Symbiosis* 66 ISSN: 0334-5114 DOI: 10.1007/s13199-0150376-5.
- Herridge, D., Rose, I., 2000. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crop Res* 65(2):229–248. [https://doi.org/10.1016/S0378-4290\(99\)00089-1](https://doi.org/10.1016/S0378-4290(99)00089-1)
- Herridge, D. F., 1982. Legume Nitrogen fixation. In *Methods to measure legume nitrogen fixation.* Workshop Handbook 1994.NWFP. Agriculture University Peshawar, Pakistan. Pp 38.
- Ibibijen, J., Urquiaga, S., Isruaili M., Alves. B.J. and Boddey, R.M., 1996. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans *Phaseolus vulgaris*. *New phytology* 134: 353 – 360
- Klogo, P., Ofori, J.K., Amaglo, H., 2015. Soybean (*Glycine max* (L) Merrill) promiscuity reaction to indigenous bradyrhizobia inoculation in some Ghanaian soils. *Int. J. Sci. Tech. Res.* 4, 306–313. <http://dx.doi.org/10.1016/j.apsoil.2005.06.008>. ISSN: 09291393.
- Nwosu, J., Olubiyi, M. R. . Aladele, S. E, Apuyor, E. B. Okere, A. U. Lawal, A. I. Afolayan, G. Ojo, A. O., . Nwadike C, . Lee M-C, Nwosu E. C. 2019. Proximate and mineral Composition of selected soybean genotypes in Nigeria *Journal of plant Development* Vol.26: pp 67-76. www.plant-journal.uaic.ro doi: 10.33628/jpd.2019.26.1.67
- Ojo, A, Dare, M.O., Fagbola, O., Babalola, O., 2015. Variations in infectivity of indigenous rhizobial isolates of some soils in the rainforest zone of Nigeria, *Arch. Agron. Soil Sci.* 61:3, 371-380. DOI: 10.1080/03650340.2014.933811
- Okalebo, J. R., Gathua, K. W., Woome, P. L., 1993. Laboratory methods of soil and plant analysis: A working manual. Tropical Soil Biology and Fertility Programme.
- Okogun, J. A., Sanginga, N., 2003. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agro ecologi-

- cal zone of Nigeria? *Biol. Fertil. Soils* 38, 26-31.
- Osunde, A., Gwam, S., Bala, A., Sanginga, N., Okogun, J., 2003. Responses to rhizobial inoculation by two promiscuous soybean cultivars in soils of the Southern Guinea savanna zone of Nigeria. *Biol. Fertil. Soils* 37, 274–279. <http://dx.doi.org/10.1007/s00374-003-0611-8>. (ISSN: 0178-2762).
- Ronner, E., Franke, A.C., Vanlauwe, B., Dianda, M., Edeh, E., Ukem, B., Giller, K.E., 2016. Understanding variability in soybean yield and response to P-fertilizer and rhizobium inoculants on farmers' fields in northern Nigeria. *Field Crop Res.* 186, 133–145. <https://doi.org/10.1016/j.fcr.2015.10.023>.
- Roughley, R. J., Gemell, L. G., Thompson, J. A. and Brockwell, J. 1993. The number of *Bradyrhizobium species* (Lupinus) applied to seed and its effect on rhizosphere, colonization, nodulation and yield of Lupin. *Soil Biology and Biochemistry*. 25: 1453 – 1458.
- Sanginga, N., Woome, P.L., 2010. Integrated soil fertility management in Africa: principles, practices and developmental process (p. 13). Tropical soil biology and fertility Institute of the International Centre for tropical agriculture (TSBF-CIAT), Nairobi, pp 263
- Sanginga, N., Abaidoo, R., Dashiell, K., Carsky, R.J., Okogun, A., 1996. Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. *Appl. Soil Ecol.* 3, 215–224. [http://dx.doi.org/10.1016/0929-1393\(95\)00089-5](http://dx.doi.org/10.1016/0929-1393(95)00089-5).
- Sanginga, N., Lyasse, O., Singh, B. B., 2000. Phosphorus use, efficiency and nitrogen balance of cowpea breeding lines in a low P soil of derived savanna zone in West Africa. *Plant Soil* 220, 119 – 128.
- Sanginga, N., Thottappilly, G., Dashiell, K., 2000. Effectiveness of rhizobia nodulating recent promiscuous soybean selection in the moist savanna of Nigeria. *Soil Biol. Biochem.* 32, 127 – 133.
- SAS., 2003. SAS/STAT .Guide for personal computers, Version 9.1 Edition, 1028 pp Cary, NC. SAS. Institute Inc.
- Smyth, A. J., Montgomery, R. F., 1962. Soils and land use in Central Western Nigeria. Ibadan Nigeria. Govt. of western Nigeria (Pub.) Ibadan 1962.
- Somasegaran, P., Hohen, H. J., 1994. Handbook for Rhizobia: Method in Legume-Rhizobium Technology. University of Hawaii NifTAL Project 1000 Holomua Road Paia, Hi96779 – 9744 USA, 450 pp.
- Thies, J.E., Singleton, P.W., Bohlool, B.B., 1991. Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. *Appl. Environ. Microbiol.* 57, 29–37 (ISSN: 0099-2240).
- Thies, J.E., Bohlool, B.B., Singleton, P.W., 1992. Environmental effects on competition for nodule occupancy between introduced and indigenous rhizobia and among introduced strains. *Can. J. Microbiol.* 38, 493–500. <http://dx.doi.org/10.1139/m92081>. (ISSN: 0008-4166).
- USDA, 2006. A basic system of soil classification for making and interpreting soil survey Agriculture Handbook 436, Natural resources conservation service USDA www3.interscience.wiley.com/journal/18981858/abstract.
- Soybean Vanlauwe B., Hungria M., Kanampiu F., Giller K.E. (2019). The role of legumes in the sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for the future. *Agric. Ecosyst. Environ.* 284, (2019) 106583 <https://doi.org/10.1016/j.agee.2019.106583>
- Vazquez, M. M., Barera, J. M. and Azcon, R. 2002. Influence of *Arbuscular mycorrhizae* and a genetical modified strains of *Sinorhizobium* on regrowth, nitrate reductase activity and protein content in shoots and roots of *Medicago sativa* as affected by nitrogen concentrations. *Soil Biology and Biochemistry* 34: 899 – 905.
- Watkin, E. L. J., O'Hara, G. W. and Glenn, A. R. 1997. Calcium and acid stress interact to affect the growth of *Rhizobium leguminosarium biovar trifolii*. *Soil Biology and Biochemistry* 29: 1427 – 1432.