Nigerian Journal of Soil Science

Journal homepage:www.soilsjournalnigeria.com

Assessment of the responsiveness of soybean varieties to *Bradyrhizobium japonicum* inoculation in Ogbomoso, SouthWest Nigeria

Olaboopo, M. T. and Kolawole, G. O. *

Department of Crop Production and Soil Science, PMB 4000, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

ARTICLE INFO

Article history:
Received May 12, 2020
Received in revised form August 17 2020
Accepted August 28, 2020
Available online September 7, 2020

Keywords:

Bradyrhizobium japonicum,
Rhizobium
Inoculation
nitrogen fixation
Soybean
varieties.

Corresponding Author's E-mail Address:

ogkolawole@lautech.edu.ng. 08037198801

https://doi.org/10.36265/njss.2020.300302

ISSN-1597-4488

ABSTRACT

Soybean varieties may respond differently to rhizobium inoculation, for improved yield and nitrogen fixation. Therefore, responses of ten soybean varieties to rhizobium were evaluated in 2018 at the Ladoke Akintola University of Technology, Ogbomoso, Nigeria, to determine if rhizobium will improve the performance of the varieties. The soybean varieties (main plot) and rhizobium (+ or -; subplot) were arranged as split-plot in Completely Randomized Design with five replications. Data were collected on plant height, root, shoot, pod and seed dry weights and nitrogen fixation. Data were subjected to Analysis of Variance, and the treatment means were compared using the Least Significance Difference at 5% probability level. Generally, the non-inoculated plants were taller than inoculated ones. The soybean varieties exhibited pronounced variation in plant height without rhizobium inoculation. Variety TGX 2004-10F was the tallest while variety TGX 1485-1D was the shortest. However, with rhizobium inoculation variety TGX 2010-12F was the tallest throughout the sampling period while variety TGX 1485-1D was still the shortest. Rhizobium inoculation had no significant effect on the shoot and root weights. On the average, variety TGX 2004-10F had the highest shoot (28.8 g/pot), and root (6.2 g/pot) weights and variety TGX 1485-1D produced the lowest shoot weight (9.5 g/pot). Varieties TGX 2008-2F and TGX 2008-4F had the lowest root weight (2.5 g/pot). Variety TGX 2010-12F was the most responsive to rhizobium in terms of the shoot (33.7% increase) and root (44.1% increase) weights. In comparison, variety TGX 2008-4F had the most negative response (-57.2% decrease) and (-100% decrease) in shoot and root weights, respectively. Rhizobium inoculation increased seed weight of almost all the varieties (9.4% - 35.4%). Rhizobium inoculation significantly enhanced nitrogen fixed. However, the varieties exhibited differences in N fixed in response to rhizobium inoculation (range -28.3% to 48.7%).

In conclusion, *Bradyrhizobium japonicum* inoculation has the potential to improve shoot, root, seed yield and N-fixed of selected soybean varieties while other varieties may show negative response.

1.0 Introduction

Soybean (*Glycine max* (L.) Merill) is an economically significant leguminous crop globally, and it is among the main crops that are grown in Nigeria. It contains 20 - 25% edible oil, and 42 - 45% protein (Alam *et al*., 2009).). It produces significantly more protein per hectare than other leguminous crops. Soybean cultivation in Nigeria has expanded as a consequence of its nutritive and economic significance and varied domestic usage. Production is mostly done by small farm holders on farms of less than five hectares (ACET, 2013).

Soybean production is primarily constrained by reduced soil phosphorus availability (Kamara *et al*., 2007; Kolawole, 2012),diseases such as soybean rust (Twizeyimana *et al*., 2008), moisture stress (Tefera, 2011) and high cost or restricted availability of good quality inputs (fertilizer, inoculants, herbicides, pesticides (ACET, 2013)).

Nitrogen is one of the most limiting plant nutrients for crop production in West Africa (Sangakara *et al*., 2003). Most legumes, through a symbiotic relationship with rhizobia, can change the N_2 through biological nitrogen fixation (BNF) into a form utilizable for plant growth. The amount of nitrogen fixed varies according to legume species (Abdul-aziz, 2013).

The discovery of biological nitrogen fixation (BNF) in the late 19th century resulted in the potentiality of BNF as an option to inorganic N- Fertilizer in Agriculture (Bala, 2011 and N'cho *et al*., 2013) due to high cost of inorganic fertilizers. This knowledge soon led to the practice of Inoculation with early implementation achieved by transferring soil from field to field, or soil to seed before planting. However, this was rapidly replaced by the application of pure cultures on agar slants, and later as broths (Bala, 2011). Hence, rhizobia inoculants are used to convey nitrogen-fixing bacteria (collectively termed rhizobia) which have been on the commercial market for over 100 years in many developed countries (Nelson, 2004; Giller, 2008; GRDC, 2013).

The benefits of Inoculation, together with the application of lacking nutrients, mostly phosphorus vary with location and soils. Inoculating legumes with rhizobia has been used to accomplish a significant increase in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels. These gains are generally highest when the inoculated legumes are grown in nil-rhizobia or low rhizobial soils but are marginal in soils already containing a high number of compatible rhizobia (Grains Research and Development Corporation, 2013).

The varietal difference affects levels of nitrogen fixation in various legume crop species, and in some crops, particular combinations of rhizobium strain and cultivar are efficient at fixing nitrogen (Graham, 2000). There are varying reports on the interaction between variety and rhizobium strain in soybean. Thao *et al.* (2002) found a significant interaction between variety and strain on nitrogen fixation parameters whereas Munyinda *et al.* (1988) reported a non-significant interaction between soybean variety and rhizobium strain

The cost of fertilizers and environmental effects of the fertilizer required for the growth and production of soybean compared with the low cost of Inoculation and benefits of Inoculation to the soil necessitates the reason for carrying out this research, as Inoculation has the potential as an option to inorganic N-fertilizer. The objective of this study was to determine if rhizobium inoculation will bring about an increase in soybean performance and also determines which variety(s) will respond positively to Inoculation.

2.0 Materials and methods

2.1 Site description

The experiment was carried out at the screen house, Department of Crop Production and Soil Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria between March and July 2018. Ogbomoso lies on latitude 8^0 10'N and longitude 4^0 10'E in the southern Guinea savanna agro-ecological zone of Nigeria.

2.2 Soil preparation and analysis

Soil samples (0-15 cm depth) were collected at the Teaching and Research Farm with the use of a shovel. The soil was air-dried and sterilized by heating the soil at above 121^{o}C inside a metallic drum for 8 hours. The soil was sent to a laboratory for bacteria count to ensure that the sterilization was successful. Total N was analyzed using the micro-Kjeldahl method. To determine P and K, samples were wet digested with a mixture of $HClO_4$-HNO_3. Phosphorus was measured colorimetricaly by molybdate blue method in an auto-analyzer, K was measured by flame photometry. The pH was determined in 1:1 H_2O soil organic C was determined by a wet combustion method. Ca and Mg were determined with an atomic absorption spectrophotometer. The soil chemical analyses followed the procedures described by Page et al. (1982).

2.3 Treatments and experimental design

Bacteria Inoculant (NODUMAX) with polymer sticker and ten varieties of soybean (five early maturing and five medium maturing) which were sourced the from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria were used for this experiment (Table 1). The experiment was conducted using a split-plot arrangement in complete randomized design (CRD) with 5 replicates. Seeds of the soybean varieties were surface disinfected by immersing in 80% ethanol for two to three minutes, followed by immersion in a 10% sodium hypochlorite (NaClO) solution for three to four minutes and then washed with sterile distilled water several times to remove all traces of hypochlorite. The recommended rate of Inoculation (100% inoculation) by the International Institute of Tropical Agriculture (IITA) was done at the rate of 10 g of legume fix inoculant per 1 kg of seed. Ten-kilogram soil each was weighed into 100 pots of 12kg capacity. Four seeds were sown per pot and later thinned to two per pot at ten days after planting.

2.4 Cultural practices/data analysis

After planting, watering was carried out once in a day for four weeks after which it was done twice daily with the use of sterilized water. Lambda-cyhalothrin at 5ml/L when necessary was applied to control insects. Weeding was carried out as needed. Data collection was carried out at 2, 4, 6, 8, 10 and 12 weeks after sowing (WAS) on plant height, leaf count, stem girth, nodule number and dry weight, dry shoot and root weights, pod number and weight per plant and seed weight. Plant height was measured from the base to top with the aid of a measuring tape. At the same time, leaf count was done by counting the number of fully opened leaves, stem diameter was measured using a vernier caliper, and this was then used to calculate stem girth using the formula πd (π =3.14 and d= diameter measured). The number of nodules found on the root after harvest was counted and then weighed using a sensitive scale to give nodule number and nodule weight. In contrast, after harvest, the root and shoot were separated, put in properly labelled brown envelopes and oven-dried at 67^{o}C until a constant weight was obtained to give dry shoot and root weights. Amount of nitrogen fixed was also evaluated using ureide analysis with the procedure of Peoples *et al*. (1989), the formula used was:

$$RUI\ (\%) = \frac{4\ X\ \text{ureide concentration}\ (mol1^{-1})}{\text{Total N concentration}\ (mol1^{-1})}$$

RUI is The Relative Ureide Index (RUI). The factor of 4 arises from the fact that one molecule ureide contains 4 N atoms. The RUI (%) was then used to extrapolate percentage N fixed from calibration curves (Peoples *et al*. 1989).

Data recorded were subjected to Analysis of Variance (ANOVA) using SAS statistical software (2009). Significant differences were assessed at 5% level of probability, and the treatment means were separated using the Least Significance Difference procedure.

3.0 Results

3.1 Pre-sowing soil properties

The soil was near neutral in pH, low in organic carbon, nitrogen, phosphorus and sandy loam in nature (Table 2).

3.2 Plant height: Without rhizobium, the soybean varieties exhibited pronounced variation in plant height. Throughout the sampling periods, variety TGX2004-10F was the tallest while variety TGX1485-1D was the shortest between 8 – 12 weeks after sowing (WAS) (Fig.1). With rhizobium inoculation, variety TGX2010-12F was the tallest throughout the sampling period, and variety TGX1485 – 1D was still the shortest similar to what was observed for uninoculated plants (Fig. 2).

Generally, the non-inoculated plants were taller than the inoculated ones (Fig. 3). Except for varieties TGX2004-13F, TGX2008-2F, TGX2010-12F, TGX2010-15F and TGX2010-3F where the inoculated plants were slightly taller than the non-inoculated plants at 12WAS.

Shoot and root weights: The soybean varieties produced the variable shoot and root weights. On the average, variety TGX 2004-10F produced the highest shoot (28.8g/pot) and root (6.2g/pot) weights while variety TGX 1485-1D had the lowest shoot weight (9.5g/pot) and varieties TGX 2008-2F and TGX 2008-4F had the lowest root weight (2.5g/plot) (Table 4). Rhizobium inoculation had no significant effect on the shoot and root weights. However, responses of the varieties to rhizobium inoculation in terms of shoot and root weights varied (Table 4). For shoot weight, variety TGX 2010-12F was the most responsive (33.7% increase), and variety TGX 2008-4F had the most negative response (-52.7% decrease). For root weight, variety TGX 2010-12F was similarly the most responsive (44.1% increase) and variety TGX 2008-4F also had the most negative response (-100% decrease) (Table 4).

Pod and seed weights: Generally, rhizobium inoculation increased pod weight of the soybean varieties except for variety TGX 2004-10F and TGX 2008-4F, which exhibited a negative response to rhizobium inoculation (Table 3). The range of responses was from -22.9 to 27.5%. On the average, variety TGX 2007-11F had significantly higher pod weight than variety TGX 2004-10F which had the least value. Similar to the observation for pod weight, on the average, variety TGX 2007-11F had significantly higher seed weight (9.8g/pot) than variety TGX 2004- 10F (5.7g/pot), TGX 2010-15F (5.8g/pot) and variety 2008-2F (4.8g/pot) which had the least value. Rhizobium inoculation increased seed weight of almost all the varieties (9.4-36.4%) while it reduced seed weight of variety TGX 2004-10F (-5.4%) (Table 3). TGX 2010-3F was more responsive to rhizobium inoculation in terms of seed weights (36.4% increase) and pod weights (27.5% increase). In comparison, variety TGX 2004-10F had a negative response to Rhizobium inoculation in terms of the pod (-22.9%) and seed weights (-5.4%).

Nitrogen fixation: On average, the varieties were not significantly ($p \geq 0.5$) different from each other in terms of nitrogen fixed (Table 5). However, rhizobium inoculation significantly enhanced nitrogen fixed. Furthermore, the

Table 1: Information on the evaluated soybean varieties

	Maturity Period (days)	Potential Yield (kg/ha)	Maturity Class	Source
TGX 2010 - 15F	90 - 100	2237	Early	IITA IBADAN
TGX 1485 – 1D	90 – 100	2237	Early	IITA IBADAN
TGX 2010 – 3F	90 – 100	2268	Early	IITA IBADAN
TGX 2004 – 3F	90 - 100	2197	Early	IITA IBADAN
TGX 2004 – 13F	90 - 100	2013	Early	IITA IBADAN
TGX 2004 – 10F	101 – 110	1818	Medium	IITA IBADAN
TGX 2008 – 2F	101 – 110	2237	Medium	IITA IBADAN
TGX 2007 – 11F	101 – 110	2207	Medium	IITA IBADAN
TGX 2010 – 12F	101 – 110	1870	Medium	IITA IBADAN
TGX 2008 – 4F	101 – 110	1507	Medium	IITA IBADAN

Table 2: Results of Pre-cropping soil analysis

Parameter	Value
pH (H_2O)	6.8
Organic carbon (g kg^{-1})	0.4
Nitrogen (g kg^{-1})	0.1
Phosphorus (mg kg^{-1})	5.5
Exchangeable cations (cmol kg^{-1})	
Calcium	2.1
Magnesium	0.5
Potassium	0.2
ECEC	2.8
Sand (g kg^{-1})	780
Silt (g kg^{-1})	90
Clay (g kg^{-1})	130
Textural class	Sandy loam

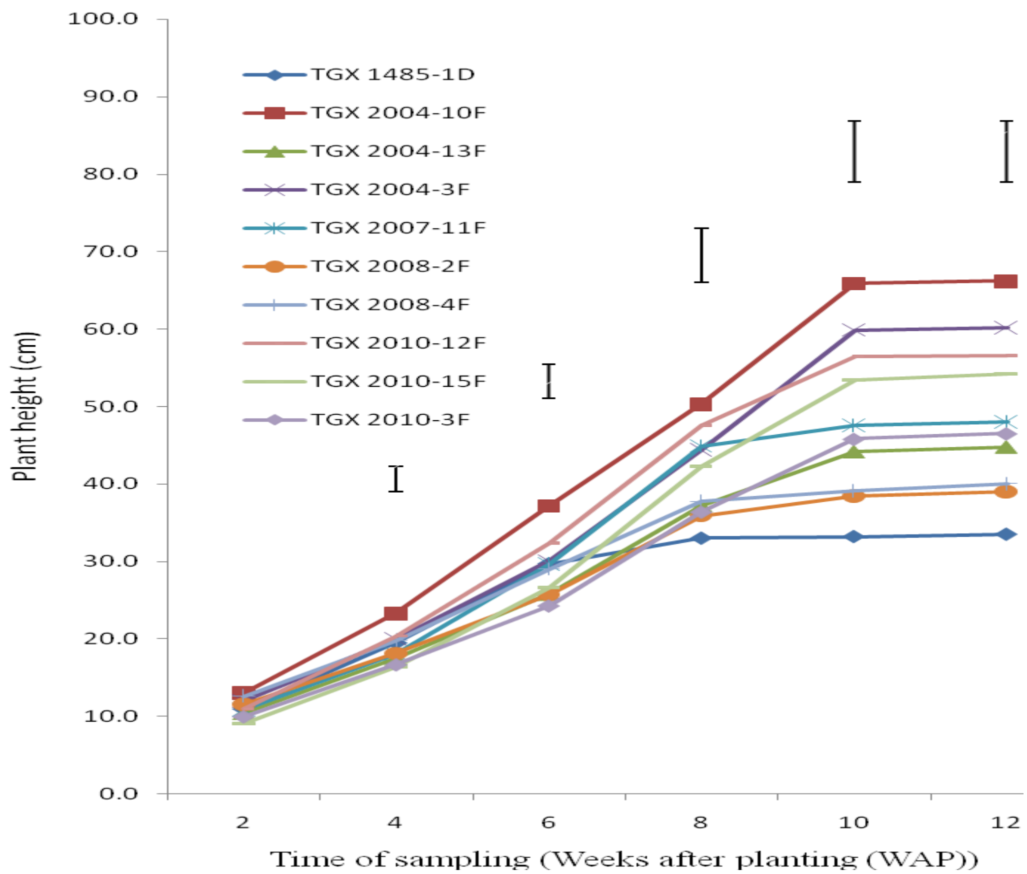

Figure 1: Heights of uninoculated soybean varieties in Ogbomoso, 2018 Legend
Bar I = LSD (0.05)

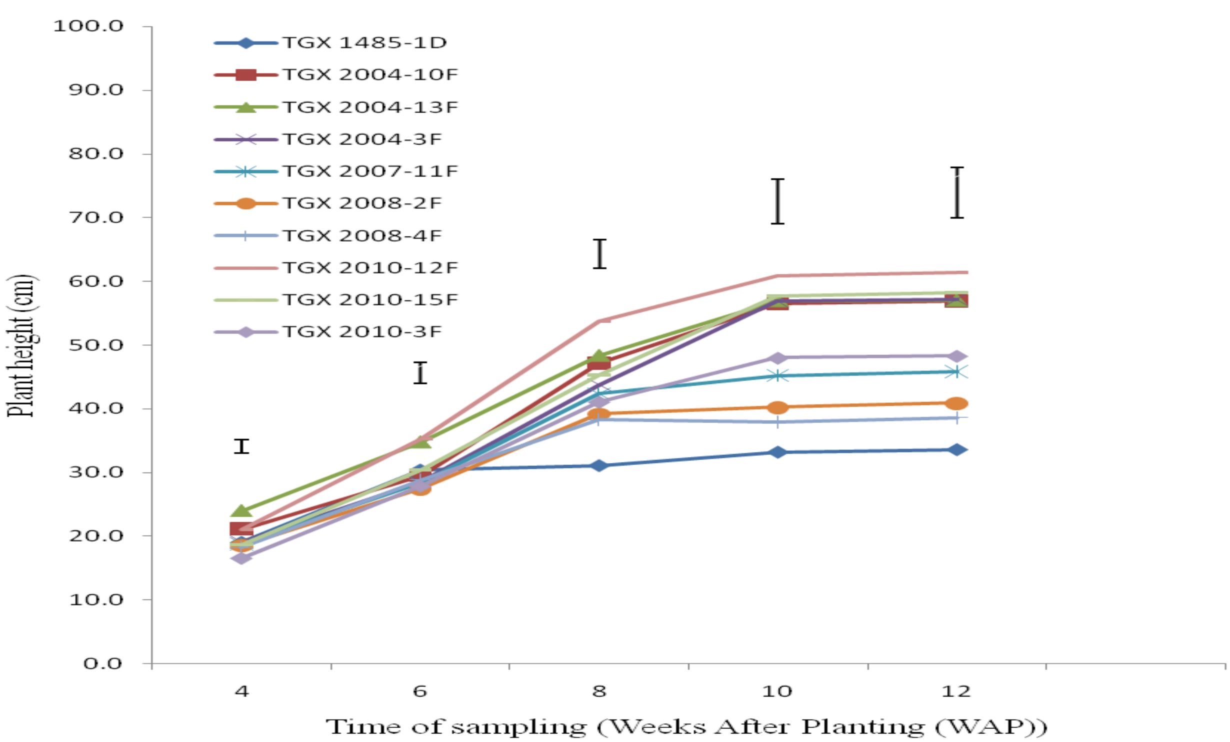

Figure 2: Effects of Rhizobium inoculation on heights of soybean varieties in Ogbomoso, 2018
Legend

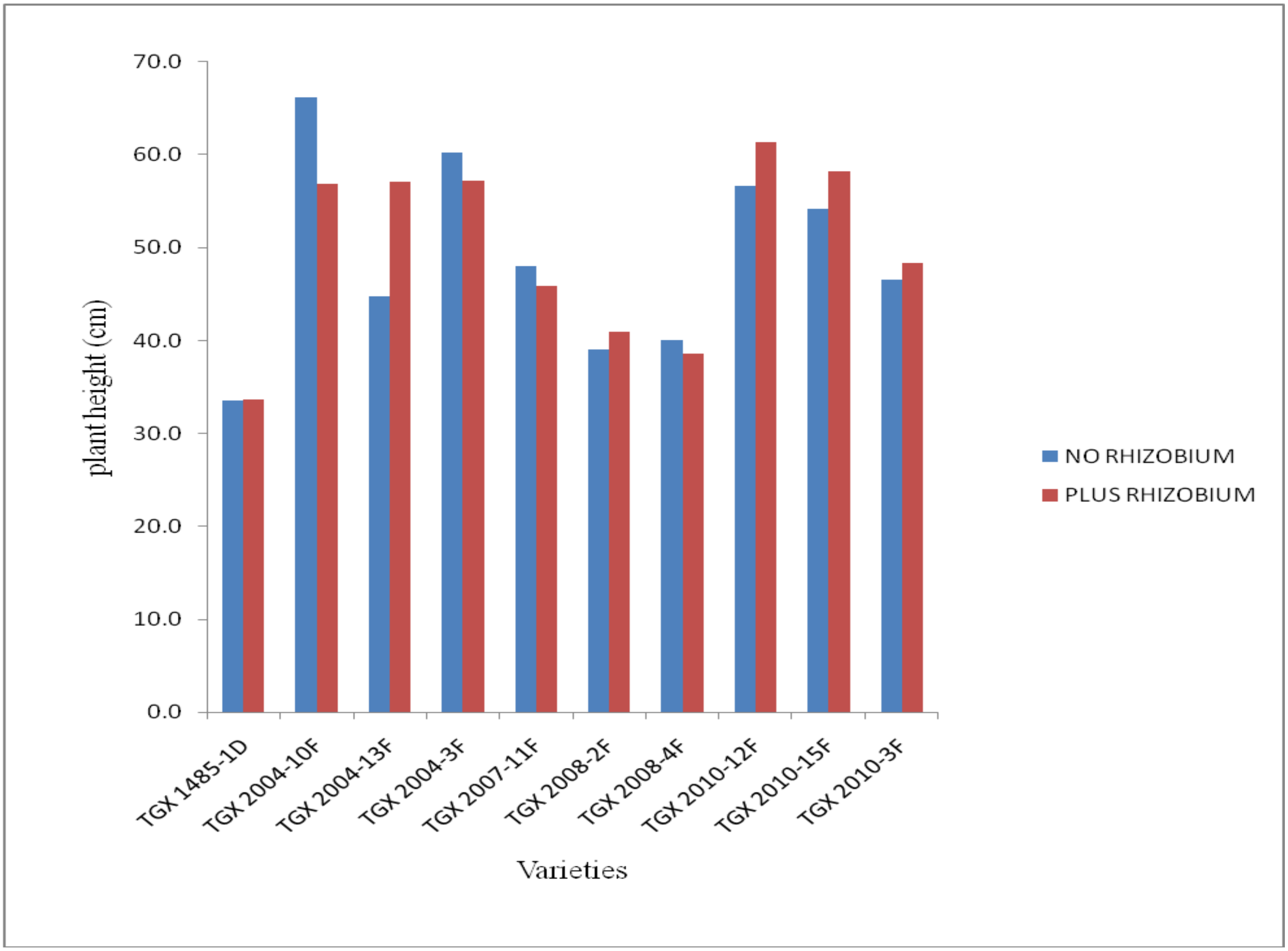

Figure 3: Heights of inoculated and non-inoculated soybean varieties in Ogbomoso at 12 weeks after planting

Table 3: Effects of rhizobium inoculation on the yield of soybean varieties in Ogbomoso, 2018

	Seed weight (g/pot)				**Pod weight (g/pot)**			
	Rhizobium				Rhizobium			
Variety	minus	Plus	%Δ	Variety mean	minus	Plus	%Δ	Variety Mean
TGX 1485-1D	5.8	6.4	10.3	6.1	11.3	12.4	9.7	11.9
TGX 2004-10F	5.9	5.6	-5.1	5.7	11.8	9.6	-18.6	10.7
TGX 2004-13F	7.4	9.6	29.7	8.5	11.4	14.8	29.8	13.1
TGX 2004-3F	7.4	10.5	41.9	9	12.8	17.1	33.6	14.9
TGX 2007-11F	8.5	11.2	31.8	9.8	14.8	18.4	24.3	16.6
TGX 2008-2F	4.5	5.1	13.3	4.8	6.6	9	36.4	7.8
TGX 2008-4F	7.2	8.4	16.7	7.8	15.8	12.9	-18.4	14.3
TGX 2010-12F	6.9	8.7	26.1	7.8	11.6	15	29.3	13.3
TGX 2010-15F	5.5	6.2	12.7	5.8	10.2	12.5	22.5	11.4
TGX 2010-3F	5.6	8.8	57.1	7.2	11.6	16	37.9	13.8
Rhizobium Mean	6.5	8			11.8	13.8		
LSD Variety	3.4				5.5			
LSD (0.05)Rhizobium	1.2				1.9			
LSD V X R	ns				ns			

Table 4: Effects of rhizobium inoculation on biomass yield of soybean varieties in Ogbomoso, 2018

	Shoot dry weight (g/pot)				Root dry weight (g/pot)			
	Rhizobium				Rhizobium			
Variety	minus	plus	%Δ	Variety mean	minus	plus	%Δ	Variety mean
TGX 1485-1D	9.0	9.9	10.0	9.5	3.5	3.9	11.4	3.7
TGX 2004-10F	31.7	26.0	-18.0	28.8	8.1	4.4	-45.7	6.2
TGX 2004-13F	10.2	10.9	6.9	10.5	2.7	2.6	3.7	2.7
TGX 2004-3F	12.2	16.9	38.5	14.5	4.3	4.8	11.6	4.6
TGX 2007-11F	19.4	18.6	-4.1	19.0	3.4	3.7	8.8	3.6
TGX 2008-2F	10.1	13.4	32.7	12.0	2.4	2.6	8.3	2.5
TGX 2008-4F	17.1	11.2	-34.5	14.0	3.4	1.7	-50.0	2.5
TGX 2010-12F	16.5	24.9	50.9	20.7	3.3	5.9	78.8	4.6
TGX 2010-15F	20.4	27.3	33.8	23.9	5.6	4.5	-19.6	5.0
TGX 2010-3F	18.6	16.5	-11.3	17.6	3.9	5.7	46.2	4.8
Rhizobium Mean	16.5	17.6			4.1	4.0		
LSD Variety	5.0				1.0			
LSD(0.05) Rhizobium	ns				ns			
LSD V X R	ns				1.6			

Table 5: Effects of rhizobium inoculation on nitrogen fixation of soybean varieties in Ogbomoso, 2018

	Rhizobium			
Variety	Minus	plus	%Δ	Variety Mean
TGX 1485-1D	77.1	66.0	-11.1	71.6
TGX 2004-10F	49.1	89.0	39.9	69.0
TGX 2004-13F	61.9	86.9	25	74.4
TGX 2004-3F	32.1	78.0	45.9	55.1
TGX 2007-11F	87.3	59.0	-28.3	73.2
TGX 2008-2F	76.7	68.6	-8.1	72.7
TGX 2008-4F	60.0	82.3	22.3	71.2
TGX 2010-12F	77.4	82.9	5.5	80.2
TGX 2010-15F	36.0	84.7	48.7	60.4
TGX 2010-3F	54.2	82.5	28.3	68.3
Rhizobium Mean	61.2	78.0		
LSD Variety	ns			
LSD(0.05) Rhizobium	8.5			
LSD V X R	27.0			

varieties exhibited pronounced differences in nitrogen fixed in response to rhizobium inoculation (Range -28.3 to 48.7 %) (Table 5). Varieties TGX 2007-11F, TGX 1485-1D and TGX 2008-2F showed a negative response to Rhizobium inoculation in terms of nitrogen fixed.

4.0 Discussion

The significant variation observed among the soybean varieties for some of the measured traits in this study is an indication of the presence of inherent genetic variation for these traits among the evaluated varieties. The non-significant responses of soybean varieties to rhizobium inoculation for plant height, root and shoot dry weights observed in this study agrees with the findings of previous researchers (Ghasem *et al*., 2015; Adeyeye *et al*., 2017) who observed that soybean inoculation treatment with bacteria *Bradyrhizobium japonicum* had no significant effect on plant height. They ascribed the reason for the insignificant effects of Inoculation to sufficient nitrogen being released from the organic matter or biological antagonism from other microorganisms indigenous to the soil used. However, in the present study, the N level in the soil was low, and there was no biological antagonism from other microorganisms indigenous to the soil used consequent upon the sterilization treatment. In contrast with the current findings, Maresha and Kibebew (2017) and Lamptey *et al.* (2014) reported that inoculated plants were more established than those in un-inoculated plots and this could have been due to a greater vigour exhibited by such plants as a result of their better growth due to Rhizobium inoculations other than the genetic and environmental factors.

The significant increase in seed yield reported as a result of rhizobium inoculation is an indication that Inoculation with *Bradyrhizobium japonicum* influenced the seed yield of soybean. This could have occurred as a result of the ready availability of soil nitrogen as a result of the symbiosis efficiency between soybean and *B. japonicum*. This was corroborated by the findings of Maresha and Kibebew (2017) which stated that seed inoculation of *B. japonicum* alone significantly increased mean seed yield per plant by 18.7% as compared to the un-inoculated control treatment. They attributed the increase to increased nodulation through a symbiosis between soybean and *B. japonicum,* which resulted in more N_2-fixation that leads to increased yield.

The percentage of nitrogen fixed by the soybean plants was significantly affected by rhizobium inoculation. The amount of nitrogen fixed for the inoculated plants was higher than uninoculated plants; this reveals that the addition of rhizobium effectively increased the amount of nitrogen fixed. This may not be farfetched, as the inoculated plants would have commenced nitrogen fixation earlier as a result of earlier nodulation occurrence due to the bacteria action than in the uninoculated plants. This is in line with the findings of Lamptey *et al.* (2014), who reported that rhizobium inoculation in soybean resulted in higher nitrogen fixation. The reason was ascribed to the presence of nucleic acid (NA) enzyme produced by *B. japonicum,* which increased nitrogen fixation in root nodules by reducing atmospheric N_2 to NH_4^+ for plant root absorption.

The interaction between the rhizobium inoculation and soybean varieties evaluated that was found to be significant for some parameters measured showed that some varieties responded differently, with some varieties responding positively with an increase in the measured parameters while some varieties responded negatively.

5.0 Conclusion

Results showed that the evaluated soybean varieties expressed differential performance for some of the observed traits, signifying the existence of inherent variability among them. Bradyrhizobium japonicum inoculant has the potential to improve seed yield and N-fixed of some soybean varieties while others (TGX 2008-4F, TGX 2007-11F, TGX 1485-1D and TGS 2004-10F) may show negative response.

5.1 Acknowledgements

The authors wish to thank the International Institute of Tropical Agriculture, Ibadan, Nigeria who supplied the soybean varieties and the commercial legume inoculant, Nodumax.

References

ACET (2013). Africa's soybean agro-processing opportunity. African Center for Economic Transformation. http://acetforafrica.org/wp-content/uploads/2014/08/ Soybean-Dalberg. Pdf, Accra. Accessed on 27/7/2018.

Adeyeye, A.S., Togun, A.O., Olaniyan, A.B., and Akanbi, W.B. (2017). Effect of Fertilizer and Rhizobium Inoculation on Growth and Yield of Soybean Variety (Glycine max L. Merrill). *Advances in Crop Science and Technology* 5: 255-260

Alam, M. A., Siddiqua, A., Chowdhury, M. A., and Prodhan, M. Y. (2009). Nodulation, yield and quality of soybean as influenced by integrated nutrient management. Bangladesh Agricultural University 7(2): 229–234

Bala, A. (2011). Update on inoculant production by cooperating laboratories. Milestone reference number: 3.4.3. N_2Africa October 2011. 8pp

Ghasem, S., Hamid R. M., and Hamid R. F. (2015). Effect Inoculation of Soybean Cultivars with bacteria *Rhizobium japonicum* in Sistan. *Biological Forum – An International Journal* **7**: 554-557

Giller, K. E., 2008. The successful intensification of smallholder farming in Zimbabwe. LEISA Magazine No. 24. 2 - June 2008.

Graham, P. H. (2000). "Nitrogen transformations," in *Hand Book of Soil Science*, M. E. Summer, Ed., p. C-141, CRC Press, London, UK.

GRDC (Grains Research and Development Corporation), Australia. (2013). Rhizobial inoculants fact sheet. Available at www.coretext.com.au. January 2013. http://www.grdc.com.au/~/media/B943F697AF9A406ABBA20E136FDB7DC4.ashx Accessed on 24/9/2018.

International Institute of Tropical Agriculture. (1982). Automated and semi-automated methods for soil and plant analysis. Manual Series No. 7. Ibadan (Nigeria): International Institute of Tropical Agriculture.

Kamara, A.Y., Abaidoo, R., Kwari, J., Omoigui, L. (2007). Influence of phosphorus application on growth and yield of soybean genotypes in the tropical savannas of northeast Nigeria. Archives of Agronomy and Soil Science **53**, 539–552.

Kolawole, G.O. (2012). Effect of phosphorus fertilizer application on the performance of maize/soybean intercrop in the southern Guinea savanna of Nigeria. Archives of Agronomy and Soil Science **58**, 189–198

Lamptey, S., Ahiabor, B. D. K., Yeboah, S., and Osei, D. (2014). Effect of rhizobium inoculants and reproductive growth stages on shoot biomass and yield of soybean (*Glycine max* (l.) Merril). Journal of Agricultural Science; Vol. **6**, No. 5

Mahamood, J., Abayomi, Y. A., and Aduloju M. O. (2009). Comparative growth and grain yield responses of soybean genotypes to phosphorous fertilizer application. African Journal of Biotechnology Vol. **8** (6), pp. 1030-1036. Available online at http://www.academicjournals.org/AJB

Masresha, A. T., and Kibebew, K. (2017). Effects of Rhizobium, Nitrogen and Phosphorus Fertilizers on Growth, Nodulation, Yield and Yield Attributes of Soybean at Pawe Northwestern Ethiopia. International Journal of Microbiology and Biotechnology. Vol. **2**, No. 1, pp. 34-42.

Munyinda, K., Karamanos, R. E. Legg, J. O., and Sanogho, S. (1988). "Nitrogen fixation by soybeans (*Glycine max* L.) in Zambia," *Plant and Soil*, vol. 109 (1):57–63.

N'cho, C. O., Yusuf, A. A., TamiaAma-Abina, J., Jemo, M., Clement, R., Abaidoo, R., and Savane, I. (2013). Effects of commercial microbial inoculants and foliar fertilizers on Soybean nodulation and yield in the northern Guinea Savannah of Nigeria. International *Journal of Advance Agricultural Science*, ***1***: 66-73.

Nelson, L. M., 2004. Plant growth-promoting rhizobacteria (PGPR): Prospects for new inoculants. Online. Crop Management. Doi: 10.1094/CM-2004-0301-05-RV.

Okereke, G.U., Onochie, C.C., Onokwo, A.U., Onyeagba, E., and Elejindu, G.O. (2004). The response of introduced Bradyrhizobium strains infecting a promiscuous soybean cultivar. *World Journal of Microbiology of Biological* 16 (1): 43-48.

Page, A.L., Miller, R.H., Keeney, D.R. (1982). Methods of soil analysis, part 2. 2nd edition. Chemical and microbiological properties. Madison (WI): ASA and SSSA.

Sangakara, U. R., Richner., W., Schnelder, M. K., Stamp,

P. (2003). Impact of intercropping beans (Phaseolus vulgaris L.) and sun hemp (crotalaria juncea L.) on growth, yields and nitrogen fixation of maize (Zea mays L.) grown in the humid and tropics during the minor season. **48**:233-238.

Tairo, V.E. And Ndakidemi, A.P. 2013. Yields and economic benefits of soybean (*Glycine max* L.) as affected by *Bradyrhizobium japonicum* inoculation and phosphorus supplementation. *American Journal of Research Communication*, 1(11): 159-172.

Tefera, H. (2011). Breeding for promiscuous soybeans at IITA. In: Sudaric, A. (Ed.), Soybean—Molecular Aspects of Breeding. Dr Aleksandra Sudaric (Ed.), Intech, Rijeka, Croatia.

Thao, T. Y., Singleton, P. W., and Herridge, D. (2002) "Inoculation responses of soybean and liquid inoculants as an alternative to peat based inoculants," in *Proceedings of the ACIAR Proceedings109e on Inoculants and Nitrogen Fixation of Legumes in Vietnam*, pp. 67–74, Oil Plant Institute of Vietnam.

Twizeyimana, M., Ojiambo, P.S., Ikotun, T., Ladipo, J.L., Hartman, G.L., and Bandyopadhyay, R. (2008). Evaluation of soybean germplasm for resistance to soybean rust (Phakopsorapachyrhizi) in Nigeria. Plant Disease. **92**, 947–952.