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Symbiotic Properties of Rhizobia Isolates Obtained from Birnin Gwari and Shika in Kaduna State, Northern Guinea Savanna Zone of Nigeria

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Abstract

A pot experiment was set up in the screen house of the School of Agriculture and Agricultural Technology, Minna. The experiment consists of 2 factors, namely nitrogen sources and soybean varieties. The nitrogen sources are 0 Kg N ha^{-1}, 100 kg N ha^{-1}, USDA110, and rhizobia isolate obtained from soils of Birnin gwari and Shika in Kaduna State (Bg 1-2, Bg 4-2, Bg 5 and Sk 6-3). These treatments were replicated three times and arranged in a Completely Randomized Design (CRD). Before the commencement of the experiment, coarse sand was obtained from the river sand, washed thoroughly to remove sediments, dirt, and dissolved salts, and then autoclaved at 121^{0}C and pressure of -15 bar to destroy microbes. After that, poly pots were filled with 2 Kg of the autoclaved sand and watered with sandsman nutrient solution to field capacity before planting four seeds of soybeans per poly pot. Five days after planting, seedlings were thinned to two, followed by the application of the treatments. Week 5 after planting, plants were harvested, and nodules were counted before oven-drying alongside shoots and roots to constant weight at 65^{0}C for three days. Dry weights of shoots of inoculated plants and that of 100 Kg N ha^{-1} plants were used for calculation of % Symbiotic Effectiveness,i.e.(Shoot Biomass of inoculant/ Shoot Biomass of +N) × 100. Data obtained were subjected to Analysis of Variance (ANOVA), and means were separated using the Least Significance Difference (LSD). Results revealed that the highest shoot biomass and percentage symbiotic effectiveness were recorded when TSB 4810 was inoculated with Bg 4-2 while the highest nodulation characteristics were recorded when TGx 1998-2E was inoculated with USDA110. The heaviest root biomass was observed with TGx 1998-2E plants inoculated with Bg5.

Keywords: Properties, Rhizobia, Soybean, Symbiotic.

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1.0 Introduction

Soybean (Glycine max. L merr) production has gained prominence in the northern guinea savanna zone of Nigeria, stimulated by demand for oil and raw material or poultry feed (Chiezey and Odunze 2009). It belongs to the large botanical family *Leguminosae* in subfamily *Papilionoideae*with other legumes like peas, beans, lentils, and peanuts (Shurtleff and Aoyagi, 2007; IITA 2009). Soybean is perhaps one of the oldest food crops of the world and ranks first among oilseed crops, and it is the richest source of plant protein known to man (Odunsanya, 2002). The total area under soybean production in Nigeria is estimated at 659,000kg ha^{-1} with a total output of 1,031,000MT, giving an average yield of 1500kg/ha with an estimated increase by 17% overestimated land area (NAERLS, 2015). Total land area under soybean cultivation in the world was 95.2 million hectares per annum, and total production was 212.6 million tons annually, according to FAO (2005). However, the production of soybean is challenged by poor crop establishment, inappropriate planting depth, use of unimproved seeds, low soil fertility, and lack of effective nodulation (Lawson and Quainaro, 2008). Facts from different sources indicate that soybeans fix atmospheric nitrogen in the range of 60 – 140 kg N ha^{-1} in one cropping season (Crouch *et al.,* 2004; Shiferaw *et al.,* 2004).

Low levels of Biological Nitrogen Fixation (BNF) is a characteristic of agricultural soil that cannot support high soybean yields without the application of inorganic nitrogen fertilizers or inoculation with soybean rhizobia (Abaidoo *et al.,* 2007).

Nitrogen is generally low in the soil. It is usually found to be a most deficient essential element in the cultivated soil of the world because soils have little capacity to retain oxidized forms of nitrogen due to ease loss through drainage, volatilization, denitrification, and heavy plant uptake. According to Okereke *et al.* (2005), lack of effective nodulation and also unavailability of suitable soybean varieties are among the causes of poor yields. Therefore, there is a wide gap between the crop's potential yield and harvested yields. Also, the problem of soil fertility in Nigeria due to infertility of soil makes the yield of soybean production to reduce, which therefore leads to food insecurity because of the reduction in yield. The introduced inoculated strains may sometimes not be effective because of environmental factors. Some strains may not adapt to environmental conditions, because of differences in the climate of their originated place and introduced place, therefore, makes the inoculant ineffective Heavy use of inorganic fertilizer in the soil tends to reduce the soil productivity which leads to soil productivity decline thereby results in low production of soybean.

The benefit gotten from the use of Rhizobium inoculants shows that farmers can save enough money by using effective inoculants on the farm. Seeds inoculants with beneficial rhizobia could be an alternative for the use of expensive commercial nitrogen fertilizer and realization of optimal productivity in the legume (Hussain *et al.,*2011). The use of highly effective inoculants will increase the yield of soybean and thereby increases food security. Sometimes soil may not have enough rhizobia for soybean; in that case, a soybean inoculant could help add beneficial bacteria back to the soil. Rhizobia inoculation can soil fertility improvement in cereal-based cropping systems in the Guinea Savanna Agro-Ecology (Carskey *et al.,* 1996) since the bacteria can fix about 300kg ha^{-1} atmospheric N leading to increased grain biomass yields (Keyser and Li, 1992). Therefore the use of highly effective inoculant will increase the yield of soybean and increases food security in Nigeria.

The objectives of this study were to determine to shoot biomass accumulation, nodule weights, and numbers of soybean varieties as a result of inoculation and to qualify the amount of nitrogen fixed through % symbiotic effectiveness

2.0 Materials and methods

2.1 Description of Study Area

This experiment was carried out at the greenhouse of the School of Agriculture and Agricultural Technology, Federal University of Technology, Minna State. Minna is situated in Southern Guinea Savannah vegetation zone of Nigeria, between latitude 9° 36' 54.86'' N and longitude 6° 32' 51.94´´E. Climate of Minna is sub-humid with a mean annual rainfall of about 1284 mm and a distinct dry season of about 5 months duration occurring from November to March. The mean temperature remains high throughout the year, about 33.5°C, particularly in March and June (Ojanuga, 2018).

The rhizobia isolate used for the experiment were obtained from Birnin-gwari and Shika. Both locations are situated in Kaduna with latitude 10° 40'0''N and longitude 6° 32' 0''E, latitude 11° 12' 0'' N and longitude7° 33' 0''E respectively; they are at the Northern part of Nigeria's high plains.

2.2 Treatment and Experimental Design

The experiment was laid out in a completely randomized design (CRD). The factors were soybean varieties and N sources. The soybean varieties were three: TSB4810, TGx1998-2E, and TGx1904-6FF treated with 0 Kg N ha^{-1}, 100 Kg N ha^{-1}, USDSA110, and rhizobia isolate obtained from Birnin-gwari and Shika (Bg5, Bg1-2, Bg4-2, SK6-3). All the treatments were replicated three times.

Sharp sand was collected, washed at least 15times to remove all soil particles, dirt, and nutrients which may be present in the sand. The sand was then sterilized in the laboratory using an autoclave machine at a temperature of 121°C for 20minutes to kill all the soil microbes which may be present and care was taken to avoid the sand from contamination. Polythene pots were arranged in a Completely Randomized Design (CRD). Polypots with a capacity of 2.5kg were used. The Polypots were filled with 2kg of sterilized sand. The poly pots were then fertilized with 0.176g of urea and labeled +N (i.e. 100kg N ha^{-1}).

2.2.1 Sandman Nutrient Preparation

Sandman's nutrient was used for the growth of soybean. It consists of calcium solution iron solution and micronutrient solution.

The calcium solution

Solution a was prepared by dissolving KNO_3 (0.2g) and $CaSO_4$ (2.5g) in 1000ml of distilled water, 200ml of calcium solution was added to the sand before planting.

Solution b was obtained by dissolving KCl (0.149g×60), $MgSO_4.H_2O$ (0.493g×60), and K_2HPO_4 (0.348g×60) in 1000mls of distilled water and 5ml of micronutrients and iron solution into 60litres of distilled water. Solution b was used to water the plant continuously throughout the experimental period.

The iron solution was prepared by dissolving $FeSO4.H_2O$ (5g) and Citric acid (5g) in 1000ml of distilled water.*Micronutrient solution* was obtained by dissolving $CUSO_4.5H_20$ (0,157g), $ZnSO_4.7H_2O$ (0.44g), $MnSO_4.7H_20$ (3.076g), $(NH_4)_6$. $Mo_7O_{24}.H_2O$ (0.02 g) and H_3Bo_3 (2.26g).

2.2.2 Yeast Mannitol Broth (YMB) Composition (Vincent, 1970)

Yeast mannitol broth in slant bottles was inoculated with native rhizobia strains isolated from Birnin-gwari (Bg5, Bg1-2, and Bg4-2) and Shika (Sk6-2) in Kaduna State. Yeast mannitol broth contains mannitol (10g), K_2HPO_4 (0.5g), $MgSO_4.7H_20$ (0.2g), NaCl (0.1g) and $CaCO_3$ (1g) in 1000ml of distilled water. Each of the isolates was cultured on YMB in a slant bottle for at least 3days at 25 – 28°C in the incubator. After which plants were inoculated just immediately after thinning at the rate of 5 ml of broth culture per plant using a fresh syringe for each isolate.

2.3 Planting and Crop Management

Seeds were washed with hypo soap and distilled water to sterilize the seeds. Before planting, Watering the sand was done using 200ml calcium solution of sandman nutrient solution.

After that, seeds were planted at the rate of four seeds per poly pot and thinned to two seedlings one week after planting. After planting, seedlings were watered at a 2days interval with nutrient solution till harvest.

2.4 Harvesting and Tissue Sampling for symbiotic Effectiveness Percentage

The plants were harvested at 5 weeks after planting. The shoots were cut using a clean, sharp knife at 1cm above the soil surface. The pots were emptied, and soils were washed to separate the roots and nodules. Nodules fresh weight, nodule number, and fresh shoot weights were recorded per plant. Fresh shoot and fresh nodules were oven-dried at 70°Cfor for 72hours to obtain the dry weight. Symbiotic effectiveness (%) was derived as follows:

Symbiotic Effectiveness (S.E) %= (Shoot Biomass of inoculant/ Shoot Biomass of +N) × 100

2.5 Statistical Analysis

All the data obtained from the experiment were subjected to Analysis of Variance (ANOVA) at a 5% level of significance. The means of treatment effects were separated by using Least Significant Difference (LSD).

3.0 Results

3.1 Main Effect of N-Sources and Soybean Varieties on Symbiotic Properties of Rhizobia Isolates.

Table 1 shows the main effect of N-sources and soybean varieties on the symbiotic properties of soybean. N source affected all the symbiotic properties at a 1% level of significance. Except for nodule number and nodule weights, variety affected symbiotic properties significantly even at 1% of the level of significance. The interaction between N source and variety significantly affected shoot biomass, root biomass, nodule number, nodule weight, and symbiotic effectiveness at a 1% level of significance.

3.1.1 Inoculation between N-Sources and Soybean Varieties on Shoot Biomass (g plant^{-1})

Table 2 shows the effect of the interaction of N-sources and soybean varieties on shoot biomass (gplant^{-1}). The heaviest shoot biomass (2.41 g plant^{-1}) was recorded when the TSB4810 variety was inoculated with Bg4-2 while the lowest of (0.68 g plant^{-1}) was recorded when TSB4810 was inoculated with Sk6-3. TGx1998-2E and TGx1904-6F produced their highest shoot biomass values when they were inoculated with 100 kg N ha^{-1} as urea while the TSB4810 variety recorded their highest shoot biomass values when they were treated with Bg4-2. Averagely, the TSB4810 variety recorded the highest shoot biomass, followed by TGx1998-2E and TGx1904-6F in that sequence. Averagely, plants inoculated with Bg4-2 recorded the highest shoot biomass.

3.1.2 Effect of Interaction between N-Sources and Soybean Varieties on Root Biomass(g plant^{-1})

Table .1 Main Effect of N Sources and Varieties on Symbiotic Properties of Rhizobia Isolates

Treatments	**Shoot Biomass (g plant^{-1})**	**Root Biomass (g plant^{-1})**	**Nodule Number (plant^{-1})**	**Nodule Weight (plant^{-1})**	**Symbiotic effectiveness (%)**
N-Sources					
-N (control)	1.11^{c}	0.48^{cd}	0.00^{b}	0.00^{b}	0.00^{c}
+N (urea)	1.64^{b}	0.67^{bc}	0.00^{b}	0.00^{b}	0.00^{c}
USDA110	1.00^{c}	0.69^{d}	50.00^{a}	0.08^{a}	65.05^{b}
Bg5	1.10^{c}	0.48^{cd}	0.00^{b}	0.001^{b}	71.06^{b}
Bg1-2	1.08^{c}	0.44^{d}	0.00^{b}	0.00^{b}	70.73^{b}
Bg4-5	2.12^{a}	1.49^{a}	2.00^{b}	0.01^{b}	156.99^{a}
Sk6-3	0.98^{c}	2.12^{a}	2.00^{b}	0.01^{b}	61.18^{b}
SE±	0.082**	0.068**	1.346**	0.006**	7.946**
Varieties (V)					
TSB4810	1.44^{a}	0.89^{a}	6.05^{a}	0.01^{a}	96.84^{a}
TGx1998-2E	1.28^{b}	0.55^{b}	8.62^{a}	0.02^{a}	39.23^{b}
TGx1904-6F	1.14^{b}	0.54^{b}	8.10^{a}	0.01^{a}	46.07^{b}
SE±	0.053**	0.045**	0.881	0.004	5.202**
Interaction					
N*V	**	**	**	**	**

Means with a different letter(s) indicated in the columns are significantly different (p≤0.05)
** Highly significant at (p≤0.01)

Table.2 Interaction between N-Sources and Soybean Varieties on Shoot Biomass (g plant^{-1})

		Varieties	
N- Sources	**TSB4810**	**TGx1998-2E**	**TGx1904-6F**
-N (control)	1.00^{hi}	1.29^{d}	1.03^{hi}
+N (urea)	1.21^{def}	2.06^{a}	1.67^{c}
USDA110	0.92^{i}	1.11^{e-h}	0.98^{hi}
Bg5	1.04^{ghi}	1.22^{de}	1.04^{ghi}
Bg1-2	1.11^{e-h}	1.09^{e-h}	1.05^{f-i}
Bg4-2	2.41^{a}	1.20^{d-g}	1.01^{h-i}
Sk6-3	0.68^{j}	1.01^{hi}	1.023^{de}
SE±	0.142		

Means with a different letter(s) indicated in the columns are significantly different (p≤0.05)

Table.3 shows the effect of the interaction of N-sources and soybean varieties on root biomass (g plant^{-1}). TSB4810 variety recorded the heaviest root biomass when inoculated with USDA110 and the lowest when inoculated with SK6-3. Conversely, TGx1998-2E recorded the highest root biomass when inoculated with Bg5 and the lowest when inoculated with Bg1-2. On the other hand, the TGx1904-6F variety rerecorded the heaviest root biomass when inoculated with Sk6-3. Generally, the heaviest root biomass was recorded when TGx1904-6F was inoculated with Bg4-2 while the lowest root biomass was observed when TGx1904-6F was treated with Sk6-3.

3.1.3 Effect of Interaction between N-Sources and Soybean Varieties on Nodule Number (Plant^{-1})

Table 3 Interaction between N-Sources and Soybean Varieties on Root biomass (g plant^{-1})

		Varieties	
N-Sources	**TSB4810**	**TGx1998-2E**	**TGx1904-6F**
-N(control)	0.460^{ef}	0.506^{ef}	0.463^{ef}
+N(urea)	0.446^{fg}	0.811^{be}	0.765^{bc}
USDA110	0.811^{be}	0.765^{be}	0.513^{ef}
Bg5	0.680^{cd}	0.880^{be}	0.430^{fg}
Bg1-2	0.586^{de}	0.429^{fg}	0.416^{fg}
Bg4-2	0.459^{ef}	0.438^{fg}	3.614^{a}
Sk6-3	0.423^{fg}	0.444^{fg}	0.323^{g}
SE±	0.118		

Means with a different letter(s) indicated in the columns are significantly different ($p \leq 0.05$)

Table.4 shows that the interaction between N-sources and soybean varieties affected the Nodule number of plants. Plants treated with control and 100kg N ha^{-1} did not nodulate. In addition to control and 100kg N ha^{-1} inoculation of TSB4810, TGx1998-2E and TGx1904-6F with Bg1-2 and Sk6-3 did not form a nodule. The highest nodule number of 60 was observed when TGx1998-2E was treated with USDA110. The lowest nodule number of 1 was observed when TGx1998-2E was inoculated with Bg4-2 and also when TGx1904-6F was treated with Bg5.

3.1.4 Effect of Interaction between N-Sources and Soybean Varieties on Nodule Weight (g plant^{-1})

Table4 Interaction between N-Sources and Soybean Varieties on Nodule Number (plant^{-1})

		Varieties	
N-Sources	**TSB4810**	**TGx1998-2E**	**TGx1904-6F**
-N(control)	0.00^{d}	0.00^{d}	0.00^{d}
+N(urea)	0.00^{d}	0.00^{d}	0.00^{d}
USDA110	35.33^{c}	60.00^{a}	55.67^{b}
Bg5	0.00^{d}	0.00^{d}	1.00^{d}
Bg1-2	0.00^{d}	0.00^{d}	1.00^{d}
Bg4-2	1.00^{d}	1.00^{d}	0.00^{d}
Sk6-3	1.00^{d}	0.00^{d}	0.00^{d}
SE±	2.331		

Means with a different letter(s) indicated in the columns are significantly different ($p \leq 0.05$)

Table 5 shows the effect of interaction between variety and N sources on nodule weight (g plant^{-1}). The highest nodule weight of 0.13g plant^{-1} was recorded when TGx1998-2E was inoculated with rhizobia USDA110 followed by the nodule weight of TSB4810 and TGx1904-6F inoculation with USDA110. Averagely, nodule weight was heaviest when plants were inoculated with USDA110, followed by the nodule weight of plants inoculated with Bg4-2.

3.1.5 Effect of Interaction between N-Sources and Soybean Varieties on Symbiotic Effectiveness Percentage (S.E %)

Table 5 Interaction between N-Sources and Soybean Varieties on Nodule Weight (g plant^{-1})

		Varieties	
N-Sources	TSB4810	TGx1998-2E	TGx1904-6F
-N(control)	0.00^{c}	0.00^{c}	0.00^{c}
+N(urea)	0.00^{c}	0.00^{c}	0.00^{c}
USDA110	0.06^{b}	0.13^{a}	0.06^{b}
Bg5	0.00^{c}	0.00^{c}	0.03^{bc}
Bg1-2	0.00^{c}	0.00^{c}	0.01^{c}
Bg4-2	0.02^{c}	0.01^{c}	0.00^{c}
Sk6-3	0.02^{c}	0.00^{c}	0.00^{c}
SE±	0.011		

Means with a different letter(s) indicated in the columns are significantly different ($p \leq 0.05$)

Table.6 shows the effect of interaction between N-sources and soybean varieties on percentage symbiotic effectiveness. Inoculation of variety TSB4810 with rhizobia isolate Bg4-2 gave the highest percentage symbiotic effectiveness of (199.17%), followed by inoculation of the same TSB4810 with Bg1-2 (96.1%). The lowest percentage of symbiotic effectiveness was observed when TGx1997-2E was inoculated with SK6-3 (48.50%). Averagely, the percentage of symbiotic effectiveness was highest when plants were inoculated with Bg4-2 and lowest when they were inoculated with SK6-3.

4.0 Discussion

Plants supplied with 100 Kg-N did not nodulate because they were not inoculated with rhizobium. Nodules are formed as a result of infection of the susceptible host by rhizobium (Shu-jie *et al*., 2007). Regardless of the variety, inoculation with USDA110 gave a significantly higher number of nodules compared to other treatments as a result of its superior infectivity. Averagely, plants treated with Bg5, Bg1-2, Bg4-2, and Sk6-3 did not form a nodule. Lack of nodulation response to inoculation could be attributed to low inoculant viability, adequate soil mineral N, incompatibility of the inoculant strain with specific grain legumes, or the presence of highly competitive native rhizobia that restricted occupancy of the nodules by the inoculant strains (Chemining'wa *et al.,*2007). In this case, the absence of nodules could be due to insufficient or loss of inoculant viability or the incompatibility of the rhizobium isolate Bg5, Bg1-2, Bg4-2, and Sk6-3 with the varieties in question (Chemining'wa *et al.,*2007). It was also observed that except for Bg5 and Bg1-2, inoculation of TSB4810 and TGx1998-2E with rhizobium isolates significantly increased nodule weight more than the control. Nodules that are statistically heavier than the nodules of control plants could be classified as useful while those that are otherwise as ineffective. This classification was adopted from (Osei *et al*., 2011) who classified shoot biomass of inoculated plants that were not statistically different from those of the control plants as a reflection of ineffective symbiosis. The dry weight of nodules formed by rhizobia strain USDA110 on TSB4810, TGx1998-2E and TGx1904-6F were the heaviest (3.79, 11.14, and 8.33 g plant^{-1}respectively). No doubt, the nodules formed as a result of inoculation with USDA110 were most likely more effective than those of the rhizobium isolates, followed by the nodules formed as a result of inoculation with Bg4-2. The non-significant difference in shoot biomass observed between plants treated with the Bg4-2 and Bg5 suggests that

Table 6 Interaction between N-Sources and Soybean Varieties on Symbiotic Effectiveness Percentage (S.E)

		Varieties	
N-Sources	TSB4810	TGx1998-2E	TGx1904-6F
-N(control)	0.00^{f}	0.00^{f}	0.00^{f}
+N(urea)	0.00^{f}	0.00^{f}	0.00^{f}
USDA110	78.45^{bc}	54.55^{e}	62.16^{de}
Bg5	90.87^{bc}	60.14^{de}	62.16^{de}
Bg1-2	96.09^{b}	52.33^{e}	63.77^{cde}
Bg4-2	199.17^{a}	48.50^{e}	58.84^{de}
Sk6-3	59.46^{e}	48.50^{e}	75.57^{bcd}
SE±	13.736		

Means with a different letter(s) indicated in the columns are significantly different ($p \leq 0.05$)

these isolates may not be different genetically. Uzoma *et al.,* (Pers. Com.) reported that Bg5and Bg4-2 were 88% similar to Rhizobium Azibense strain obtained from Tunisian soils, respectively. Surprisingly, Bg5 produced plants that were not statistically different in shoot biomass compared to inoculated plants. Could there be the existence of some form of genetic similarities between Bg5 and USDA110? Whether there be or not, both rhizobia entities produced the highest shoot biomass indicating that the physiology of plants was the most affected as a result of inoculation with these rhizobia entities through the improvement of photosynthesis and dry matter accumulation (Hungria and Mendes, 2015; Agoyi *et al.,* 2016). Averagely, the shoot biomass of +N plants was statistically superior to that of the USDA110, and Bg5 inoculated plants signifying that USDA110 and Bg5 cannot wholly replace 100 N kg^{-1}urea especially in the cultivation of TSB4810, TGx1904-6F, and TGx1998-2E. It is, however, worthy to note that the shoot biomass observed may necessary not be a result of symbiosis. It is symbiotic when nodules are formed. In the case of inoculation with USDA110,despite the superior modulation, the shoot biomass was not superior to those of Bg inoculated plants. This suggests that the association between USDA110 and the soybean varieties were not symbiotic but parasitic. Nodules were formed at the expense of shoot formation. Plants that accumulated superior shoot biomass without nodulating suggests a non-symbiotic association between the plant and the rhizobium or a form of unclassified association.

Nevertheless, superior shoot biomass is an indication of nutrient use or supply efficiency (Janagard and Ebadi-Segherloo, 2016), and the mechanism by which this is achieved may not be fully understood. Rhizobia isolate Bg4-2 produced more shoot weight of 2.14 g $plant^{-1}$compared with other N sources probably because of the competitiveness, infectiveness, and compatibility of its rhizobium strain (Chemining'wa *et al.,* 2007). Rhizobium isolate Sk6-3 accumulated the highest root biomass suggesting higher accumulation of carbohydrate in the root of the plants while plants treated with –N and rhizobia isolates Sk6-3 accumulated the lowest probably because carbohydrate synthesis and accumulation were hindered. Translocation of carbohydrate from the point of synthesis (leaf) to the point of storage (root) was most likely hindered by the association of these soybean varieties with -N and rhizobia isolate Bg1-2. Averagely, Sk6 -3 that produced the lowest shoot biomass produced the highest root weight implying that the roots competed for carbohydrate; a form of source-sink competition. This is fundamentally physiological and may not necessarily be a function of the association since nodules were not formed.

4.1 Conclusion

Growth and nodulation characteristics of soybean varieties were improved by N sources compared to control. The highest shoot biomass and percentage symbiotic effectiveness were recorded when TSB4810 was inoculated with Bg4-2 while the highest nodulation characteristics were recorded when TGx1998-2E was inoculated with USDA110. The heaviest root biomass was observed with TGx1998-2E plants inoculated with Bg5.

4.2 Recommendation

In the advent of global warming as a result of climate change, a farming system that encourages minimal use of N fertilizers should be recommended. Therefore to cultivate TGx1904-6F successfully, resource-poor farmers would need to make sacrifices in inorganic N supply to the tune of 24kg N ha^{-1}because its association with Sk6-3 will supply about 76% of its N biologically. In the case of the cultivation of TGx1998-2E, inoculations with Bg5 will save the farmer 60kg N ha^{-1} as biological nitrogen fixed. The cultivation of TSB4810 may not need an initial supply of starter nitrogen since its association with the isolates, except Sk6-3 and USDA110 produced Symbiotic effectiveness percentage values greater than 90%.

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