

Effect of rhizobial inoculation on the nodulation, growth and yield of Soybean in the Savannah regions of Nigeria

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Abstract— Rhizobial inoculation is the introduction of rhizobium bacteria in a high concentration into the soil before or during the planting of crops. Field experiments were conducted during the rainy seasons of 2020, 2021, and 2022 at the Research farm of the Department of Crop Sciences, Faculty of Agriculture, University of Abuja, Gwagwalada, the Southern Guinea Savanna of Nigeria. The study aimed to analyse the effect of Bradyrhizobium japonicum *inoculation on the nodulation, growth and yield parameters in Soybean. The treatments consisted of three Soybean varieties, namely TGX 1485-1D (early maturing), TGX 1448–2E (medium maturing), and TGX1987–10F (medium maturing) and Two levels of inoculant (control, with inoculant), The experiment was laid out in a randomized complete block design (RCBD), with three replicates and 54 plots. Agronomic and cultural practices were duly observed, and data were collected for growth and yield parameters. Data were subjected to analysis of variance (ANOVA) using the 'agricolae' package in the R Statistical Programme (R version 4.2.2.), T-test was conducted using the SPSS statistical package (Version 23), and the Duncan Multiple Range Test (DMRT) and Standard error (SE) were used to separate significant means at (P* \leq *0.05). Results showed that for all varieties of Soybean observed, the inoculated had the best performance (2685kg/ha, 2842.3kg/ha, 3038.5Kg/ha for TGX 1485-1D, TGX 1448–2E and TGX1987–10F respectively best compared to the control.*

Keywords— Rhizobium, Inoculation, Nodulation, Yield, and Soybean.

I. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a leguminous plant grown annually. It is from the Fabaceae or Papilionacae family (Britannica, 2022). It is an important oilseed crop, with an estimated global oilseed output of over 50%. It is also a promising pulse crop nutritionally desirable because it provides essential nutrients for vegetarian diets and, due to its usage in producing chemical goods and plant proteins, is a highly sought-after grain legume due to its versatile usage in human food, animal feed, and industrial products, including biodiesel production (Kiwia *et al*., 2022). It is the most significant bean in the global economy and is suggested to solve the severe protein and oil shortages worldwide (Jagadesh *et al*., 2020). The demand for soybean as a source of oil for biodiesel production is steadily increasing, and this trend is expected to continue even more rapidly, driven by factors such as volatile crude oil prices and environmental concerns associated with crude oil usage (Bello, 2015). However, despite its importance, Soybean (Glycine max L.) is highly susceptible to drought, nitrogen and phosphorus insufficiencies (Tehulie *et al*., 2021). This is due to their shallow and fibrous root system, making Soybeans particularly susceptible to nutrient deficiencies (Soares *et al*., 2021). Nitrogen, an essential nutrient, though present in the atmosphere in large amounts, cannot be fixed by plants, thus requiring microorganisms like bacteria to help fix it into the soil for plant absorption (Haxim, 2021). Inadequate nitrogen levels in the soil can impede the vital photosynthesis process, ultimately affecting the growth and development of soybean plants. Nitrogen fixation is particularly crucial during the R5 stage, which marks the

onset of seed growth and the highest demand for nitrogen to produce vital proteins that store in the developing seed. Given this, it is imperative to supplement the soil with additional nutrients to achieve optimal yield (Faozi *et al*., 2019).

Therefore, for soybean plants to achieve optimal growth, development, and the realization of their genetic potential, a diverse range of macro- and micronutrients is necessary as soybean plants significantly demand nitrogen (N) and phosphorus (P) to produce high-quality seeds hence it is essential to utilize inoculation through the use of plantspecific nitrogen-fixing bacteria and the best suited bacterial strain for Soybean plant is *Rhizobium japonicum* (Agrilearner, 2023). Hermann Hellriegel (1831-1895), a renowned agricultural chemist from Germany, made a significant discovery regarding leguminous plants. He found that these plants can capture atmospheric nitrogen and restore it as ammonium in the soil. This process, now commonly referred to as Nitrogen fixation, was unveiled by Hellriegel's research (Fernandes and Ravi, 2023). *Biological nitrogen fixation* is a fascinating process that occurs when the rhizobium invades the roots of compatible host legume plants. This causes unique structures called nodules to grow on the roots. The biological nitrogen fixation (BNF) process in nature sets up the supply of reactive nitrogen needed for protein synthesis and plant growth. Understanding BNF is important for making many decisions about how to grow legumes (Iantcheva and Naydenova, 2020). The interaction between bacteria and plants is chemotactic. Plants chemically attract the bacteria to their roots, activating nodule formation. Nitrogen is assimilated in the form of nitrate and ammonia. Nitrate reductase turns nitrate into nitrite, and nitrite reductase turns nitrite into ammonia (Haxim, 2021). Rhizobial inoculation is the introduction of rhizobium bacteria into the soil before or during the planting of soybeans. One way to achieve this is by utilizing an inoculant with a significant concentration of rhizobium bacteria, as Soretire *et al*. (2020) suggested. Rhizobium bacteria, capable of fixing nitrogen, play a crucial role in nutrient fixation. It works to synthesize the unique nitrogenase enzyme required for nitrogen fixation. Due to symbiosis with legume roots, leguminous plants convert gaseous nitrogen from the atmosphere into inorganic compounds, subsequently serving as a readily available crop source. This nitrogen is essential for forming chlorophyll molecules and for plant photosynthesis. Inadequate nitrogen levels can lead to symptoms such as yellowing of vegetation, reduced density, wilting, and overall growth retardation.

Microbial inoculants, also known as biofertilizers, Via their biological processes, can mobilize essential nutritional elements in the soil from non-usable to usable forms for crop plants. Their biological activity in the rhizosphere is often used as an environmentally friendly way to reduce chemical fertilizers, improve soil fertility, and increase crop yield (Annadurai and Srinivasan, 2019). When rhizobium bacteria invade the roots of compatible host plants, a nodule, which resembles a tumour, is created; it is one of the most significant mutualistic connections between microbes and plants. The nitrogenfixing bacteria in the nodule can transform atmospheric nitrogen into ammonia, providing the nitrogen needed for plant and bacterial development. Nitrogen fixation in plant nodules is fundamental to maintaining soil fertility for agricultural purposes and increasing crop yields. As highlighted by Iantcheva and Naydenova (2020), this practice is crucial in ensuring the efficiency and effectiveness of farming activities. By harnessing this method, farmers can guarantee a sustainable nitrogen supply to their crops, thus optimizing their yields by capitalizing on the gains of nitrogen fixation in plant nodules as part of their soil management strategies to enhance productivity and sustainability. Insufficient or inactive rhizobia would negatively affect nodulation, leading to poorer yields in Soybean production as they require large amounts of nitrogen when flowering and setting pods.

II. MATERIALS AND METHODS 2.1 Description of the study area

The field experiment was conducted at the Research farm of the Department of Crop Sciences, Faculty of Agriculture, University of Abuja, Gwagwalada, Federal Capital Territory. The experiment was conducted during the 2020, 2021, and 2022 rainy seasons. The research farm is situated at Lat 9° 32N and Long 50° 10E, covering an area of 11,824 hectares. Gwagwalada is located in the North Central Zone of the Southern Guinea Savanna in Nigeria, bordered by Niger, Nasarawa, Kogi, and Kaduna (Ishaya and Abaje, 2009). The region experiences a humid tropical climate characterized by distinct seasonal patterns, including periods of harmattan between December and early February, a dry season from February to April, and a rainy season from April to November. The average yearly temperature in the area ranges from 30 to 37°C, with the highest temperatures occurring in March. The mean annual rainfall is approximately 1,650 mm, with around 60% of this precipitation occurring between July and September (Ishaya and Abaje, 2009).

2.2 Experimental materials

Three varieties comprising one early maturing and two medium maturing varieties were used for the study. The names of the varieties were TGX 1485-1D (early), TGX 1448 – 2E (medium), and TGX 1987-10F (medium). The varieties were sourced from the International Institute of Tropical Agriculture, Ibadan (IITA). The inoculant *Bradyrhizobium japonicum* (Nodumax) was used. Nodumax, a legume inoculant specifically designed for soybean, created through collaborative efforts between the IITA Business Incubation Platform and the N2Africa Project. This inoculant comprised more than 1 x 10⁹ *Bradyrhizobium japonicum* strain USDA 110 per gram and was conveniently packaged in an alumino-laminate bag. The packaging also includes Gum Arabic adhesive and detailed user instructions (N2Africa, 2015).

2.3 Treatments and experimental design

The experimental treatments were laid in a $3 \times 2 \times 3$ factorial of three soybean varieties (TGX 1485-1D, TGX 1448- 2E and TGX 1987-10F**)** , two levels of inoculant (without, with inoculant), and three levels of Phosphorus fertilizer (0kg/ha, 20kg/ha, 40kg/ha) in a Randomized Complete Block Design with three replicates. Each replicate consisted of 18 plots, the total plots were 54 plots. The gross plot size were 3m x 3m (9m²) each, while the net plot was $2m \times 2m$ (4m²) each. A distance of 0.5m was observed between plots and 1m between replicates.

2.4 Experimental procedures and crop management

The experiment was first set up in the cropping season of 2020, this was repeated in the cropping seasons of 2021 and 2022. Planting dates for the years were $5th$ July 2020, 5th July 2021 and 5th July 2022 respectively. The field was ploughed and harrowed twice, then demarcated into plots. Each plot measured 3 x 3 m with an alley of 0.5 m between plots and 1 m between blocks. Three seeds were placed in each hole using the dibbling planting process. The holes were then covered with soil for a secure and favorable seed-soil environment. As the plants grew, the seedlings were later thinned out to maintain two plants per hole, adhering to a crop spacing of 75cm x 10cm. All important cultural practices were undertaken based on the recommendation for Soybean crop (Omoigui *et al*., 2020). The inoculation process was carried out before planting, with un-inoculated seeds planted first to prevent contamination.

The process of seed inoculation followed the procedures outlined by Omoigui *et al*. (2020) in the following manner: Clean and uninfested seeds were picked, and 1kg of these seeds was measured. The Gum Arabic contents in the packet were dissolved in 200 ml of warm water to create a solution that acts as a sticker; this solution is essential for ensuring that the applied inoculum adheres appropriately to the seeds. Next, using the recommended ratio of 1kg of soybean to 10g of inoculant, 10g of Nodumax was added to the sticker solution and mixed thoroughly to achieve a consistent mixture. The seeds were then carefully combined with the prepared mixture, ensuring they were evenly and uniformly coated with the Gum Arabic and inoculant solution. The inoculated seeds were spread out on paper, placed in a shaded area to shield them from direct sunlight; this step aimed to preserve the viability of the bacterial cells. After allowing a few hours for air drying and ensuring all the seeds were completely dry, the treated seeds were immediately sown at the appropriate rate and spacing.

2.5 Data Collected

Data were collected growth and yield parameters namely;

2.5.1 Plant height (cm)

This measurement was taken at three specific time points: 3 weeks after sowing (3WAS), (6WAS), and (9WAS). Five randomly selected plants were chosen from each plot, and their heights were measured using a meter rule. The height was measured from the ground level to the point of attachment of the uppermost (flag) leaf. The average height was then calculated for each plot.

2.5.2 Number of Leaves per plant (no)

Number of leaves per plant in each plot from five random selected tagged plants were recorded at 3WAS, 6WAS AND 9WAS and mean calculated and recorded.

2.5.3 Leaf Area index (LAI)

The leaf area index for each plot were taken at 9WAS. It was calculated using

Leaf area index $=$ Leaf area

Ground cover

2.5.4 Yield (Kg/ha)

The plants from each net plot were harvested and sun dried. After drying, the plants were threshed to separate the seeds from the rest of the plant material. Finally, the seeds were winnowed to remove any remaining debris or chaff. The dry weights of the grains adjusted to 13% moisture were taken and converted to kilogram per hectare for analysis.

2.5.5 Number of nodules (no)

The number of nodules per plant was assessed at the flowering/pod formation stage (11WAS). Five randomly selected plants were chosen from each plot, and the number of nodules was recorded. This was done by uprooting the entire plant carefully using a fork to ensure

the roots and nodules remained intact. The entire root system was exposed during uprooting to prevent any nodule loss. The soil adhering to the roots was removed by soaking the soil and root ball in a barrel filled with water, followed by thorough rinsing in a separate water-filled barrel. The total number of nodules per plant was recorded for the five sampled plants, and the mean value was calculated (Dabessa and Tana*,* 2021).

2.5.6 Effective nodules (no)

The colour of the nodules was visually examined to determine their effectiveness. Five randomly selected plants from each treatment were dug up using a spade. The plants were washed with clean water to separate the roots from the soil. The nodules were carefully detached from the roots and counted. The nodules were cut open using a knife. A hand lens was used to assess their effectiveness; the Nodules that appeared reddish or pink were classified as active, while those that were white or lacked colour were considered inactive. The observations regarding the colour and effectiveness of the nodules were recorded. Nodules were sampled twice the first at flowering and two weeks later (Dabessa and Tana*,* 2021).

The nodule effectiveness for the inoculated and uninoculated plots were analysed. A scoring system was used to grade the colours of the nodules (Colour score).

- The Reddish colour was scored 2
- The Pink colour was scored 1
- No colour was scored -0

An independent T-test was conducted to determine any statistically significant difference in the mean colour score for inoculated and un-inoculated plots at 9WAS and 11WAS, respectively. The 2020, 2021 and 2022 mean scores were added to get a total score. The maximum score attainable for either week (9WAS and 11WAS) was 6, and the Mean colour score, T score and p-value were recorded and evaluated.

2.6 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using the 'agricolae' package in the R Statistical Programme (R version 4.2.2), T test was conducted using the SPSS statistical package (Version 23). Duncan Multiple Range Test (DMRT) and Standard error (SE) were used to separate them at ($P \le 0.05$). Correlation coefficients was used to assess the degree of association between various traits, yield and among different yield components. Pearson's correlation coefficient formula method shown below was used for the computation (Bozokalfa *et al.,* 2010).

III. RESULTS AND DISCUSSION

3.1 Growth parameters as influenced by Soybean inoculation.

Plant height (cm) - The interaction of V x I on plant height (cm) for the mean year analysis is presented in Table 1. At 6WAS and 9WAS TGX 1987 -10F produced taller plants (45.67cm, 67.53cm) with the application of inoculant than all other treatment combinations but was comparable to TGX 1448- 2E (66.51cm, 66.09cm) with or without inoculation at 9WAS. The inoculation response on the different soybean varieties' plant height (cm) was studied. Results for the years showed that at $(P \le 0.05)$ all the varieties significantly differed in plant height compared to the control. The observed difference in plant height among soybean varieties might be attributed to inherent genotypic differences and may also be due to the rhizobia inoculation, which promotes plant growth, leading to taller plants with larger stems and more branches (Tekola *et al*., 2018). Rhizobia play a crucial role in facilitating nitrogen fixation from the air in soybean plants, which is vital for their growth and development. Ogbuehi (2020) also reported similar results, indicating that applying Rhizobium inoculants on soybean seeds increased plant height compared to untreated seeds.

Furthermore, he observed that the inoculated plants outcompeted weeds, enabling them to capture more light and other essential growth resources, thereby influencing their overall height. The findings of recent studies suggest that both inoculation and phosphorus application have the potential to promote soybean growth and development. However, it is essential to note that the impact of these practices on plant height may vary significantly based on multiple factors, such as soil type, weather conditions, and the specific type of rhizobia used. As Tekola *et al*. (2018) proposed, a thorough understanding of these factors is necessary to optimize the benefits of inoculation and phosphorus application in soybean cultivation.

Number of leaves : The interaction of V x I on the number of leaves for the mean year analysis are presented in Table 2. At 6WAS and 9WAS varieties, TGX 1987-10F at $(P \le 0.05)$ had a significantly higher number of leaves per plant (56.08, 99.17) than TGX 1485- 1D (49.26, 89.89) but produced a similar number of leaves compared to TGX 1448-2E (51.49, 94.51) with the application of the Inoculant. Ndor *et al*. (2019) conducted a similar study, demonstrating that inoculated soybeans exhibited a significantly higher number of leaves than uninoculated soybeans. On the other hand, Lambon *et al*. (2018) reported in their screen house experiment, report that rhizobia inoculation and phosphorus supplementation did not significantly influence the number of leaves and

leaf area index (LAI). However, their field experiment observed 76% improvement in number of leaves and LAI

due to inoculation compared to the control group.

Table 1: Interaction between Varieties and Inoculant on the Plant height (cm) Per Plant of Soybean in Mean years at the University Research farm, Gwagwalada, Abuja.

Means followed by the same letter(s) are not significantly different at 5% level of probability (DMRT), SE=Standard error

Table 2: Interaction between Varieties and Inoculant on the Number of Leaves per plant (no) of Soybean in Mean years at the University Research farm, Gwagwalada, Abuja

Number of Leaves Per Plant (no) Mean years Inoculant							
Varieties	Control	Inoculant	Control	Inoculant			
TGX 1485-1D	35.51d	49.26 _b	63.58e	89.89c			
TGX 1448-2E	42.78c	51.49b	69.9d	94.51 _b			
TGX 1987-10F	43.69c	56.08a	71.05d	99.17a			
1.378 $SE+$	1.229						

Means followed by the same letter(s) are not significantly different at 5% level of probability (DMRT), SE=Standard error

Leaf Area Index: The interaction of V x I on the leaf area index for the mean year analysis is presented in Table 3. The leaf area index for all varieties studied was at par and showed a higher leaf area index with the application of inoculant above control. The leaf area index showed variations in all the years of study, but in all cases, the treatments with amendments performed better than the control. This could be a result of the fact that the inoculant contains elements that are involved in various metabolic processes of Rhizobia, which in turn improved microbial efficiency, hence promoting increased growth factors; the N availability following inoculation, is essential for leaf production, as is well known in plant and crop physiology leading to the production of larger leaves which is responsible for the increased leaf area and leaf area index.

Another probable cause is the genotypic differences between the varieties. Ogbuehi (2020) conducted a study that yielded similar findings, demonstrating the significant influence of *B. japonicum* inoculation and P supplementation on the Leaf Area Index (LAI). In the glasshouse and field experiments, inoculation had notable effects compared to the control treatment. The inoculated soybean plants exhibited a significant increase in leaf area index, with a 31% and 157% improvement in the glasshouse and field experiments, respectively, compared to the control. Furthermore, applying 20, 40, and 80 kg/ha of phosphorus in combination with inoculation resulted in a substantial increase of 11%, 29%, and 44% in leaf area index compared to the control group.

Table 3: Interaction between Varieties and Inoculant on the Leaf Area Index of Soybean in Mean years at the University Research farm, Gwagwalada, Abuja

Means followed by the same letter(s) are not significantly different at 5% level of probability (DMRT), SE=Standard error

Yield: The inoculated varieties for all the years and the mean of the years showed a significant grain yield difference above the control. The interaction of V x I on the yield (Kg/ha) for the mean year analysis is presented in Table 4. All varieties showed higher yields with the application of inoculants above the control. Variety TGX 1987-10F recorded the highest yield at 3038.5kg/ha, TGX 1448-2E with 2842.3kg/ha and TGX 1485-1D with 2685kg/ha, respectively.

These results were consistent with those obtained by Mosupiemang *et al*. (2021), who reported that several yield components determine the productive potential of soybeans. Genotype x inoculation interaction significantly increased the number of pods per plant. This indicates that responses of soybean varieties differ in the number of pods and total yield based on *Bradyrhizobium spp* and the

genotype used. Adjei-Nsiah *et al*. (2022) also reported significant increases in soybean pods with the application of inoculants. They observed that the combined use of inoculant and P-fertilizer resulted in four times more pods than the control. Furthermore, Ogbuehi (2020) in his yield analysis revealed that treatment regimens significantly influenced the number of pods. Soybean with amendments of inoculant and phosphorus produced a significantly higher number of pods (85.750) than those from control plots (62.250). Variety TGX-1448 recorded the highest number of pods per plant, while TGX-1835 had the lowest number, further emphasizing the role of genetic variability in soybean production. This result was consistent with that obtained by Biswas *et al*. (2023), who reported that improving varieties in any crop is the prerequisite to increasing productivity.

Table 4: Interaction between Varieties and Inoculant on the Yield (Kg/ha) of Soybean in Mean years at the University Research farm, Gwagwalada, Abuja

Means followed by the same letter(s) are not significantly different at 5% level of probability (DMRT), SE=Standard error

Number of nodules and Effectiveness: Variety TGX 1987 -10F had the highest number of nodules (no) (22.68), while the variety TGX 1485 -1D had the lowest number of nodules (19.88) for the mean study year Table 5. An independent T-test was performed to compare the mean colour score (for nodule effectiveness) between inoculated

and uninoculated plots for the mean years shown in Table 6. At 9WAS, the mean colour score for inoculated plots was 4.84, while for the uninoculated, it was 4.22; the 11WAS mean colour score for inoculated plots was 4.11, while for the uninoculated, it was 3.46, Showing there was a significant difference between the inoculated and uninoculated plots at both 9WAS and 11WAS.

These results showed for all inoculated varieties at $(P \leq$ 0.05), there was a significant difference in the number of nodules and nodule effectiveness above the control. This confirms the report by Buernor *et al*. (2022) showing that the use of inoculation can synergistically affect nodulation and soybean yields. Inoculation can also increase the

number and effectiveness of nodules, improving the growth and development of the plant and leading to more vigorous nodules and higher yields. Based on the findings of Adjei-Nsiah *et al.* (2022), nodulation significantly increased with the simultaneous application of inoculant and P-fertilizer. Similarly, the application of inoculant alone led to an 18% increase in nodule number.

Table 5: Interaction between Varieties and Inoculant on the Number of Nodules (no) of Soybean in Mean years at the University Research farm, Gwagwalada, Abuja

Number of Nodules (no) Mean years					
Inoculant					
Varieties	Control	With Inoculant			
TGX 1485-1D	15.94d	23.81b			
TGX 1448-2E	18.08c	24.77b			
TGX 1987-10F	18.97c	26.4a			
SE_{\pm} 0.566					

Means followed by the same letter(s) are not significantly different at 5% level of probability (DMRT), SE=Standard error

Table 6: Independent T test Comparing Mean Colour score for Inoculated and Uninoculated Varieties of Soybean in mean years at the University Research farm, Gwagwalada, Abuja.

Week	Inoculated Varieties	Uninoculated Varieties	T test	P - value	Significance
At 9WAS	4.84	4.22	6.017	0.036	∗
At 11WAS	4.11	3.46	5.250	0.028	∗

At 5% level of probability

IV. CONCLUSION AND RECOMMENDATIONS

Based on the objectives and the results obtained in the current study, there is sufficient evidence showing that at (P **≤** 0.05), inoculation with *Bradyrhizobium japanicum* showed a significant positive relationship between the effect on the growth and yield parameters. This implies inoculation can be used to increase grain yields of soybeans in an economically viable manner. Showing that with inoculation, both promiscuous and nonpromiscuous varieties can get the right rhizobia strain for a symbiotic relationship, leading to more effective nodules on the roots of soybeans and invariably increased yield. It is recommended that to achieve sustained Soybean production, farmers in Sub-Saharan Africa should be encouraged to utilize inoculation due to its immense benefits as it enhances soil fertility for subsequent crops,

improves crop yields, root development, and overall plant health, is pocket-friendly, eco-friendly and positively affects grain yield.

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