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Soybean Growth Modulation Using Tomato Root Associated Plant Growth Promoting Rhizobacteria

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Abstract: Food security occurs when all individuals have constant access to adequate, secure, and nourishing food to satisfy their dietary requirements for healthy living. Soybean is a major oil crop that also serves as a food legume. Rhizobacteria that promote plant growth (PGPR) have been proven to boost legume growth and soybean development. The present study aims to determine the effects of PGPR on soybean seedlings. Two samples of tomato rhizospheric soil were obtained from a private farm in Ikpoba Okha, Edo state, Nigeria and Faculty of Agriculture, University of Benin, Nigeria. Bacterial strains were characterized morphologically and biochemically from the samples. Four isolates were identified *Pseudomonas* sp. strain 1, *Pseudomonas* sp. strain 2, *Citrobacter* sp., and *Yersinia* sp. The strains were analyzed for PGP traits like hydrogen cyanide (HCN), indole acetic acid (IAA), and ammonia production. Two isolates (*Pseudomonas* sp. strain 1 and *Citrobacter* sp.) out of the four isolates showed better plant growth promoting traits. *Pseudomonas* sp. strain 1 produced the highest level of ammonia. They both had high concentration and absorbance levels of IAA. *Pseudomonas* sp. strain 1 had a concentration level of 20.92±0.02 µg/ml and *Citrobacter* sp. had a concentration level of 18.00±0.03 µg/ml. The two species were used to grow the soybean seeds for seven days, and plant growth parameters were measured. A significant difference in growth between the seed inoculated with the bacterial treatments (*Pseudomonas* sp. strain 1, *Citrobacter* sp., a co-inoculation of both species) and the non-inoculated seeds (control) was observed. This study has shown that PGPR has positive effect on soybean seedling growth.

Keywords: *bacteria, food security, legume, seedlings*

1. Introduction

Food security is a necessary condition for mankind's existence and economic activity, including food production. Food is distinct from other necessities and production due to its relevance to living and existence. Food security is the availability and access to food (Otaha, 2013; Miladinov, 2023; Bitari *et al.*, 2024; Hassan *et al.*, 2025). A home is said to be food secure if none of its members experience famine or hunger. There is food security when everyone has physical, social, and financial access to enough wholesome food to satisfy their dietary needs (Idachaba, 2006; Burchi & De Muro, 2016).

Soybean (*Glycine max (L.) Merrill*.) is an annual legume important of the pea family (*Fabaceae*), it is also an edible seed and an important agricultural produce. As a major oil crop and a staple food legume, soybeans are among the most significant in the world (Liu *et al.*, 2008). Beyond being used in various culinary preparations and animal feed, soy oil has a wide range of industrial and residential uses. With a 53% share of all oil-seed crops, soybeans are widely used in major countries' agricultural production systems. Soybeans have several health advantages. The phytoestrogens called isoflavones, or genistein and daidzein, found in soybeans may help prevent cancer, according to some doctors and dietitians. Sales of soybeans have also grown, which is mostly attributable to the Food and Drug Administration's (FDA) recognition of soy as a recognized food that lowers cholesterol (He and Chen, 2013). Additionally, it has a high concentration of phytic acid, which has chelating and antioxidant properties (Shamsuddin, 2002). Soybean consumption is on the rise, as it ranks as the second most produced and used vegetable oil globally, making up 28% of total oil production, trailing only palm oil at 37%. Nigerian demand for chicken and edible oils continues to outpace supply despite these industries' expanding demands.

The rhizosphere of a plant contains bacteria known as rhizobacteria, which are root-associated and can affect plant development in a negative (parasitic forms), neutral, or positive way (Vessey, 2003). Generally, about 2-5% of rhizosphere bacteria are plant growth-promoting rhizobacteria (Antoun and Prevost, 2005). They work in symbiotic relationships with the plant to encourage its growth (Munees and Mulugeta, 2013). Plant growth promoting Rhizobacteria are soil bacteria that live on the root surface and are directly or indirectly engaged in encouraging plant growth and development through the creation and release of numerous regulatory compounds in the vicinity of the rhizosphere (Kloepper, 1992). They function either directly—by acquiring resources or adjusting the hormone levels in plants—or indirectly—by reducing the infections' inhibitory effects on plant growth and development when acting as biocontrol agents (Bloemberg and Lugtenberg, 2009). Based on their mechanisms, there are different classes of PGPRs and they include biofertilizers (*Azospirillum* which can be found in maize, sorghum, *Rhizobium*, *Bradyrhizobium* which can be found in soybeans and fixes Nitrogen), rhizomediators, Phytostimulators (species of *Bacillus*, *Pseudomonas*) which produce substances that aid the growth of the plant, they aid hormone synthesis like Auxins, Gibberellin (Lugtenburg and Kamilova, 2009). Plant-beneficial rhizobacteria have the potential to reduce the world's reliance on dangerous agricultural chemicals that upset agroecosystems (Munees and Mulugeta, 2013). The study sought to investigate the impact of plant growth-promoting rhizobacteria on soybean seedlings.

2. Methodology

2.1 Sample Collection

The rhizospheric soil sample of tomatoes (*Solanum lycopersicum*) was used for this experiment and the soil samples were obtained from two different locations, a private farm in Ikpoba-Okha Local Government Area, Edo state, Nigeria and the Department of Soil Sciences, Faculty of Agriculture, University of Benin, Edo State, Nigeria. The soil samples of the tomatoes obtained were collected from different portions of the farm, like the greenhouse and the open field. In the open field, a portion was collected and, in the greenhouse, a portion was collected also. The tomato plant was uprooted and the soil located around the root region was collected with a sterile instrument (trowel, spade, ramming hammer which was used to determine the depth of the soil taken) into a sterile soil sample bag while gloves were worn. In the laboratory, the soil samples were weighed with an electronic balance and sieved to get rid of other particles and debris to ensure better results while testing. The soil was placed in a cool dry place to ensure aeration and reduce the level of physiochemical change it could undergo. The soybean seeds were collected from the International Institute of Tropical Agriculture, Ibadan, Oyo state, Nigeria. Two varieties of the seeds were obtained, the Tgx-1951-3F and Tgx-1485-1D. The seeds were preserved in a sample bag and transported to Edo State where the experiment was carried out.

Figure 2.1: Tomato farm in Ikpoba Okha, Edo State

Figure 2.2: Greenhouse containing Tomato plant, Ikpoba Okha, Edo State

2.2 Microbiological Analysis of the Soil Samples

2.2.1 Preparation of Culture Media

The media used were prepared according to the manufacturer's instructions. The media used is Nutrient Agar.

2.2.1.1 Preparation of Nutrient Agar

The method for preparing nutrient agar was as directed by the manufacturer. One litre of distilled water and twenty-eight grams of nutritional agar powder were dissolved in a conical flask lined with cotton

wool and aluminium foil paper. After thorough mixing, it was autoclaved for 15 minutes at 121 degrees Celsius to sanitize it. Before aseptically distributing the medium onto Petri plates in a laminar flow, it was cooled to 45–50 ºC (El Guerrouj *et al.*, 2023).

2.2.2 Enumeration and Isolation of Microorganisms

The soil samples were weighed with the measuring scale and 1g of the sample was weighed out of the whole sample aseptically into 9 ml of distilled water in a test tube. The cap is closed tightly and shaken properly to ensure an adequate mixture of the soil sample and the water. A ten-fold serial dilution was subsequently prepared by transferring 1 ml of the sample mixed with water into a test tube containing 9 ml of distilled water as the diluent. Further serial dilutions were carried out from 10^{-1} to 10^{-2} dilutions. After serial dilution, the aliquot was transferred aseptically to sterile petri dishes. Nutrient agar was prepared and poured in aseptically. The bacterial culturing was made using the pour plate method. Between 18 to 24 hours, the plates were incubated at 37˚C room temperature. A colony counter was used to count the colonies once the bacteria had grown successfully, and the findings were recorded for each dilution count. The soil samples' total heterotrophic bacterial colony-forming units per millilitre were measured following incubation. In Nutrient agar, 0.1 ml of the suitable serially diluted samples were used to calculate the total heterotrophic bacterial count.

To create a pure culture, a single colony was found and re-streaked as the primary inoculant on the nutrient agar plate medium. Once a pure culture was obtained, the same colony was streaked onto a nutrient agar substrate. They were then incubated for 24 hrs at 37°C. The pure cultures were obtained for observation of the individual isolates and to hold further tests. The plates were counted and the cultural characteristics observed were written down. Each colony's cultural characteristics e.g., size, shape, margin, elevation, consistency, colour, and transparency are determined (Idibie *et al*., 2018).

2.2.3 Morphological Tests

On 18–24 hour cultures, Gram staining was done. On a sterile glass slide, a smear of every bacterial isolate was created, and it was either air-dried or heat-fixed with a flame. The principal stain, crystal violet (0.3%w/v), was applied and left a minute to stand. With distilled water, the stain was cleaned. After adding and letting the Gram iodine (0.4%w/v) rest for a minute, the mixture was cleaned with distilled water. After adding ethanol (95%w/v), it was let to stand for one minute. After that, it was washed with distilled water and stained for another minute using Safranin (0.4%w/v), a secondary stain. After that, it was cleaned with distilled water, much like in the previous stages, and it was examined using an oil immersion objective (x100) to check for cell form, arrangement, and gram stain. Under a microscope, the bacteria would look pink if they were Gram-negative and purple if they were Gram-positive. Additionally decided upon were the cell's configuration and form.

2.2.3.1 Biochemical Tests

Biochemical tests were carried out for further identification of the isolates. These tests include:

2.2.3.2 Catalase Tests

This test determines if the catalase enzyme is present or not. As hydrogen peroxide breaks down, free oxygen gas is released and water is formed thanks to the catalase enzyme. Placing the bacterial isolates

on a slide, a few drops of recently made 3% hydrogen peroxide were applied. Catalase is an enzyme that is present in test cultures when gas bubbles are produced; colonies of pure cultures that do not exhibit any gas generation were classified as catalase-negative (Oyelakin *et al*., 2023).

The chemical formula = $2H_2O_2 \rightarrow 2H_2O + O_2$

2.2.3.3 Oxidase Tests

The primary purpose of this test is to distinguish between *Pseudomonas* species and other rod-shaped, Gram-negative bacteria. The test culture plate's suspicious colony was rubbed with a strip of filter paper dipped into an oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride), which was made according to normal protocol. The reaction was examined for the development of colour in the cells within 20 seconds. An intense purple colouration indicated a positive result.

2.2.3.4 Urease Tests

This test determines if the test organism is capable of producing the urease enzyme, which catalyzes the conversion of urea to ammonia. Urea agar base was the media used. After autoclaving, the produced medium was allowed to cool to 50 degrees Celsius before being mixed with 5 milliliters of 40% urea solution. After being injected into the tube holding the urease reagent, the test microorganisms were cultured for 24 to 48 hours at 37 °C. Yellow becoming red-pink (fuchsia) in color, indicated a successful outcome. The chemical formula: is $NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

2.2.3.5 Indole Production Test

This is used to assess the capacity of isolated bacteria to convert the amino acid tryptophan into indole. The tryptophan was provided by the tryptone in the culture media. One liter of distilled water included five grams of commercially available tryptone water medium dissolved in it. After that, the medium was sterilised for 15 minutes at 121°C in an autoclave. A measured 4 milliliters of the medium were poured into sterile test tubes, and each test tube was then lightly inoculated with a culture that had been growing for 18 to 24 hours. Following a 24- to 48-hour incubation period at 37°C, Kovac's reagent was added to the inoculation test tubes to create a layer on top of the medium. A successful outcome was represented by a purple ring at the point where the two liquids met.

2.2.3.6 Sugar Fermentation Test

The ability of each isolate to ferment a particular sugar with the production of either gas and acid or only acid was assessed. Since most bacteria, especially Gram-negative bacteria, use different sugars as a source of carbon and energy with the formation of acid and gas or acid only, the test is used as an aid in the differentiation of bacteria. The medium for growth used was peptone water, which was prepared in a conical flask and then supplemented with phenol red. The mixture was placed in the Durham tubes that were placed into the test tubes. To sterilize the tubes and their contents, they were autoclaved for 15 minutes at 121°C. Separately, a 1% solution of sugar was prepared, and it was sterilized at 115°C for 10 minutes. Subsequently, 5 milliliters of it were aseptically introduced into the tubes containing

the indicator and peptone water. The young culture of the isolates was injected into the tubes, and they were then incubated at 37 °C. Either acid and gas production or acid-only production was seen after the incubation period of around 24 hours. Durham's tubes showed signs of gas creation and acid formation, respectively. The medium's color changed from pale green to yellow.

2.2.3.7 Citrate Utilization Test

The basis for this test is the capacity of certain organisms to grow only on citrate as a source of carbon and energy. A liter of distilled water was used to dissolve 23g of commercially available Simon's citrate medium, which was then agitated well and autoclaved for 15 minutes at 121^{o}C to sterilize it. Following the medium's dispensing into sterile test tubes, the test organism was injected and given a 24-hour incubation period at 37°C. After incubation, a rich blue color developed, indicating that the experiment was successful.

2.2.3.8 Hydrogen Sulphide Test

When a nutritional culture medium containing cysteine and sodium thiosulfate as the sulfur substrates is combined with a heavy metal salt that contains lead (Pb^{2+}) or iron (Fe^{2+}) ions as an H_2S indicator, hydrogen sulphide generation can be identified. A favourable result was shown by the medium becoming black when the isolates were added to Triple Sugar Iron Agar, which was prepared according to the manufacturer's instructions and incubated at 37 ^{o}C for 18 to 24 hours.

2.2.3.9 Coagulase Test

The purpose of this test is to distinguish between the test organism's capacity to manufacture the coagulase enzyme, which clumps red blood cells. This is mostly used to distinguish pathogenic *Staphylococci* from non-pathogenic ones. A crayon was used to divide a slide into two halves. Each portion was then covered with a loop filled with distilled water and a little bit of an 18–24 hour culture. Then drops of plasma were added to one section, allowed to stand for about 5 seconds while in the other section distilled water was added to serve as a control. Clumping indicated a positive result and the second section showed no coagulation indicated a negative result.

2.3 Plant Growth Promoting Rhizobacteria Tests

Plant growth promoting rhizobacteria tests were performed to see if the isolates have plant growth stimulating properties. The numerous tests conducted include:

2.3.1 Ammonia Production Tests

The ability of bacterial isolates to produce ammonia in peptone water was examined. In each tube, 10 ml of peptone water was used to inoculate freshly produced cultures, which were then cultured for 48–72 hours at 28±2°C. Each tube received 0.5 ml of Nessler's reagent. The test for ammonia production was positive when the color changed from brown to yellow (Kanchan *et al.*, 2018).

2.3.2 Phosphate Solubilizing Tests

Pikovskaya's agar was made, and after utilizing a pH meter to get the final composition's pH down to 7.0, the medium was autoclaved at 121°C for 15 minutes. After autoclaving, the medium was transferred to Petri dishes and left to harden. Each isolate's 25 µl broth culture was added to wells with

a diameter of eight millimeter created in the medium. A clean zone surrounding the wells on the plates, after four days of incubation at 27 °C, suggested a successful result. The phosphate solubilizing index was obtained.

PSI = Total diameter (colony + clear zone)/diameter of colony

2.3.3 Indole Acetic Acid Production Test (IAA)

IAA was quantitatively analyzed at 500 µg/ml of tryptophan. At 28±2 °C, cultures of bacteria were grown on their respective medium for 72 hours. Completely developed cultures were centrifuged for 30 minutes at 3000 rpm. The supernatant (2 ml) was combined with 4 ml of the Salkowski reagent (50 ml, 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution) and two drops of orthophosphoric acid. The emergence of pink hue signifies the creation of IAA. An optical density reading was obtained at 530 nm using a spectrophotometer (Sharma *et al*., 2012).

2.3.4 Hydrogen Cyanide Production Test

On a modified agar plate, bacteria were streaked after the nutrient broth was modified with 4.4 g glycine/l. Placing a Whatman filter paper No. 1 soaked in a solution of 0.5% picric acid and 2% sodium carbonate on the plate's ceiling. After 4 days of incubation at 28±2 °C, plates were sealed with parafilm. Production of HCN was suggested by the emergence of an orange or dark red color (Sakthivel and Karthikeyan, 2012).

2.4 Bacterial Growth and Preparation

Freshly developed cultures of the isolated bacteria were centrifuged for 300 seconds at a speed of 5000 rpm. The pellets were then cleaned in 0.85% (w/v) normal saline solution and pulverized in salt solution before being added to the solution.

2.5 Seed Germination Test

As 0.5 milliliter of sterile distilled water was added to the non-inoculated (control) plates, 0.6 milliliter of bacterial isolates were pipetted onto Petri dishes with Whatman filter paper. In the co-inoculation (combination of isolates), 0.3ml of individual isolates was pipetted, thereby making up a total of 0.6ml because there were two combinations of isolates. Soybean seeds (Tgx 1951-3F and Tgx 1485-1D) were surface sterilized in 75% alcohol and rinsed in sterile distilled water. Following that, ten seeds were added to each of the petri dishes with inoculated filter sheets, and each of the plates were gently stirred. There were three replicates of each treatment. The plates were sealed with parafilm paper and cultured for seven days in a growth chamber. The following formula was used to determine the percentage seed sprouting rate after counting the number of germinations per petri dish:

Percentage seed germination (%) = n/N x 100

The total number of seeds is N, and the number of seeds that germinate after seven days is n. Following the calculation of the percentage of seed germination, the fresh weight, taproot length, taproot breadth, shoot length, shoot width, dry weight, and number of leaves were estimated.

2.6 Statistical Analysis

Microsoft Excel and SPSS were used to evaluate the data collected for the various tests. The data were subjected to Analysis of Variance (ANOVA) and then the Duncan test to ascertain mean differences. A P-value of less than 0.05 was deemed significant.

3. Results and Discussions

Table 1 shows the total bacterial heterotrophic count from Tomato rhizospheric soil samples. The bacterial count of sample A was $4.83x10^2$ cfu/g while that of sample B was $3.67x10^2$ cfu/g. For the preliminary morphological characterizations of potential plant growth-promoting rhizobacteria, four pure bacterial colonies were isolated and characterized for their cultural traits such as size, shape, colour, margin, elevation, and transparency (**Table 2**) and also characterized for cellular morphology and characteristics using light microscopy (**Table 3**). Isolates were obtained from the two samples grown on culture plates. The isolates were named isolates 1, 2, 3, and 4 (**Table 2**). The shape of isolates 1 and 2 were irregular, isolate 3 was rhizoidal, while isolate 4 was filamentous. The colours of isolates 1 and 2 were greenish-blue, while isolate 3 was grey, and isolate 4 gave a creamy colour.

Biochemical tests were used to further identify the four bacterial colonies isolated. Three of the organisms isolated were Gram-negative while one of the isolate was Gram-positive. The four isolates discovered were *Pseudomonas* sp. Strain 1, *Pseudomonas* sp. Strain 2, *Citrobacter* sp. and *Yersinia* sp. (**Table 4**). The four organisms isolated were also screened for plant growth promoting traits. This plant's growth-promoting rhizobacteria characteristics were indole acetic acid production (IAA), ammonia production test, hydrogen cyanide (HCN) production, and phosphate solubilization. Two of the isolates performed better in the test, testing positive for HCN (**Table 7**) and producing IAA in higher concentrations (**Table 8**). Isolates were tested for phosphate solubilization. The isolates all showed positive results with clear zones on Pikovskaya's agar, then the phosphate solubilizing index was obtained. *Pseudomonas* sp. strain 1 had the highest PSI, followed by *Citrobacter* sp., the species with the least PSI was *pseudomonas* sp. strain 2. (**Tables 5 and 6**) and testing positive for ammonia production (**Table 7**). The seed germination percentage of the inoculated and non-inoculated soybean seeds is shown in **Figure 1**. **Figure 2-10** shows the effect of the isolated plant growth promoting rhizobacteria on soybean seeds, it also shows the level of significance between inoculated seeds and the non-inoculated seeds.

Table 1: Total heterotrophic bacterial count from Tomato soil samples

Total Heterotrophic Count (x 10^2cfu/g)	
Soil sample	**Bacteria**
Sample A	4.83±4.50
Sample B	3.67±2.10

Legend: Values represent mean ± standard deviation, sample A – Tomato soil from the greenhouse of a private farm, sample B – Tomato soil from Faculty of Agriculture, University of Benin, Cfu/g – Colony forming unit/gram.

Table 2: Cultural characteristics of the isolates

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Shape	Irregular	Irregular	Rhizoid	Filamentous
Size	Large	Medium	Large	Medium
Elevation	Flat	Flat	Flat	Flat
Transparency	Opaque	Opaque	Opaque	Opaque
Margin	Serrated	Undulate	Lobate	Filiform
Colour	Greenish-blue	Greenish-blue	Grey	Cream

Table 3: Microscopic characteristics of isolates

Characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram stain	-	-	+	-
Cell type	Rod	Rod	Rod	Rod
Cell arrangement	Single	Chain	Chain	Single

Legend: + = Positive, - = Negative

Table 4: Biochemical Characteristics of Isolates.

Biochemical Tests	Results			
	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Urease	-	-	-	-
Indole	-	-	-	-
Citrate	+	+	+	-
Catalase	+	+	+	+
Oxidase	+	+	-	-
H_2S	-	-	+	-
Lactose	-	-	A	-
Sucrose	-	-	+	A
Glucose	-	-	+	+
Fructose	-	-	+	A
Maltose	-	-	+	A
Sorbitol	-	-	+	A
Organism	***Pseudomonas* sp. strain 1**	***Pseudomonas* sp. strain 2**	***Citrobacter* sp.**	***Yersinia* sp.**

Legend: + = Positive, - = Negative, A = Acid, H_2S = Hydrogen sulphide

Table 5. Bacterial count for phosphate solubilization test

	Pseudomonas sp. strain 1	*Pseudomonas* sp. strain 2	*Citrobacter* sp.	*Yersinia* sp.
Colony count	17.2±1.40	31.7±0.50	23.3±2.16	34.2±3.70

Legend: Values represent mean ± standard deviation

Table 6: The effectiveness of phosphate solubilization of bacterial isolates is indicated by the phosphate solubilization index (PSI)

Isolate	Phosphate solubilization index (PSI)
Pseudomonas sp. strain 1	2.08±0.03
Pseudomonas sp. strain 2	1.24±0.08
Citrobacter spp.	1.77±0.07
Citrobacter spp.	1.31±0.09

Legend: Values represent mean ± standard deviation.

Table 7. Plant growth promoting traits: Hydrogen cyanide (HCN) and ammonia production test

Tests	*Pseudomonas* sp. strain 1	*Pseudomonas* sp. strain 2.	*Citrobacter* sp.	*Yersinia* sp.
Hydrogen Cyanide	+	–	++	–
Ammonia Production	++	+	+	-

Legend - +++ = Strong positive, ++ = Moderate positive, + = Weak positive, - = Negative

Table 8. Plant growth promoting traits: Indole Acetic Acid (IAA) Production Test at 0.5mg/ml of tryptophan

Parameters	*Pseudomonas* sp. Strain 1	*Pseudomonas* sp. Strain 2	*Citrobacter* sp.	*Yersinia* sp.
Absorbance	0.137±0.000	0.109±0.002	0.153±0.001	0.080±0.001
Concentration(µg/ml)	20.92±0.02	16.42±0.04	18.00±0.03	11.21±0.01

Legend: Values represent the mean ± the standard deviation

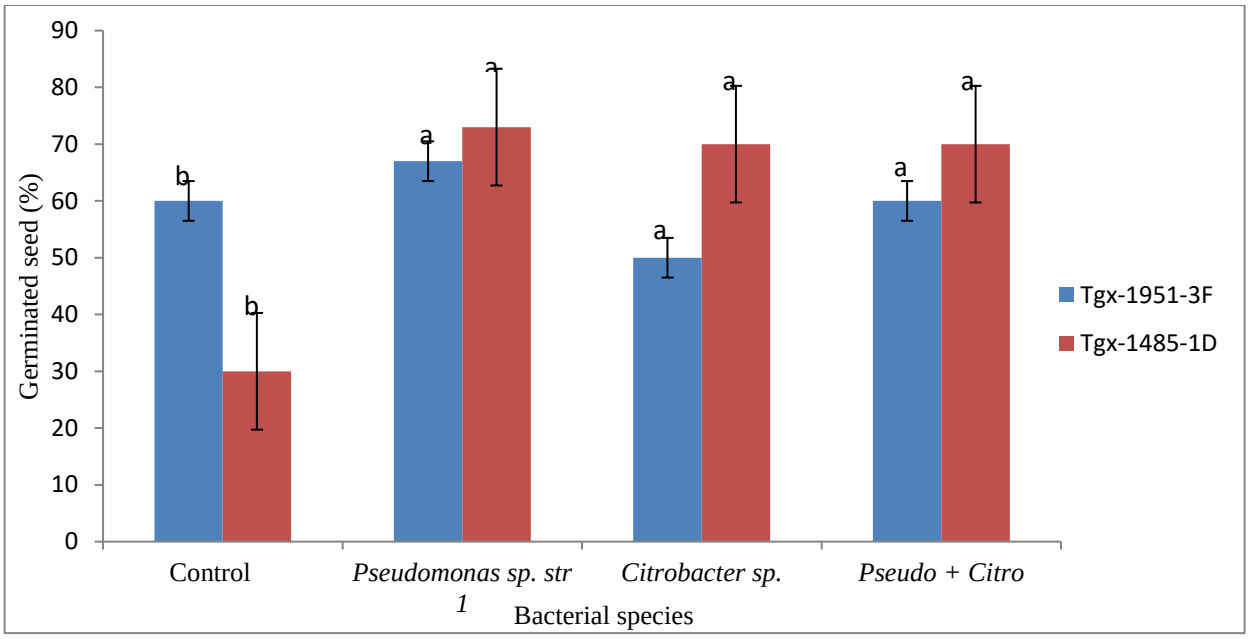

Figure 1: Germinated Tgx-1951-3F and Tgx-1485-1D soybeans seeds (%) inoculated with *Pseudomonas* sp. strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represent strain, *pseudo*. represent *Pseudomonas, Citro*. represent *Citrobacter*

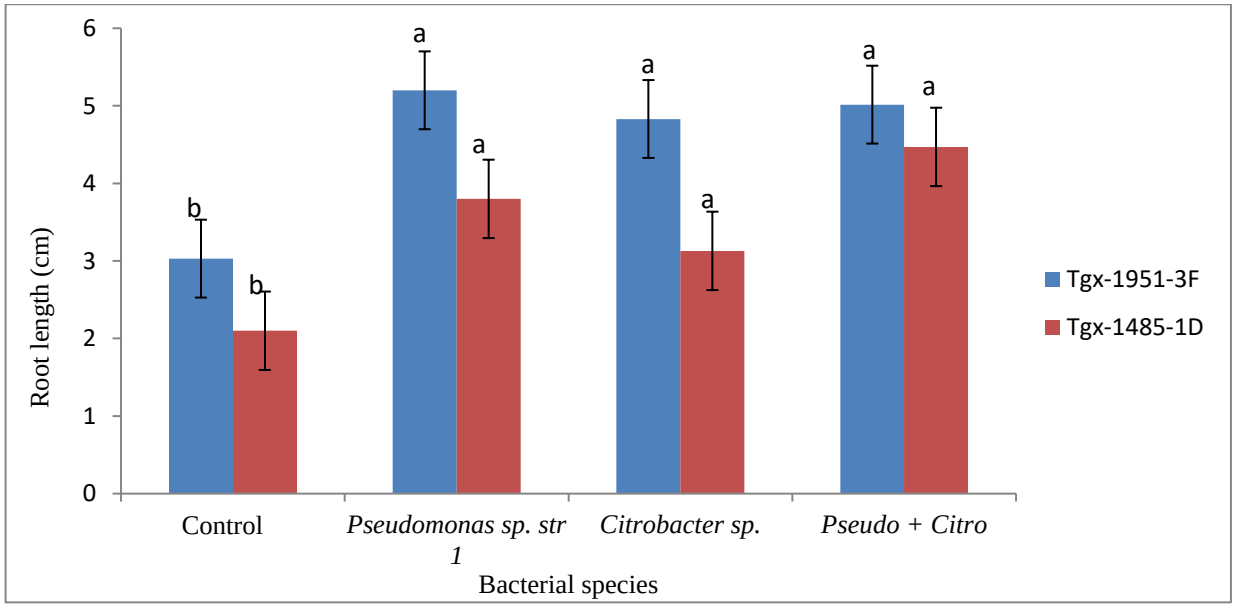

Figure 2: Root length of Tgx-1951-3F and Tgx-1485-1D soybeans seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represent strain, *pseudo*. represent *Pseudomonas, Citro*. represent *Citrobacter*

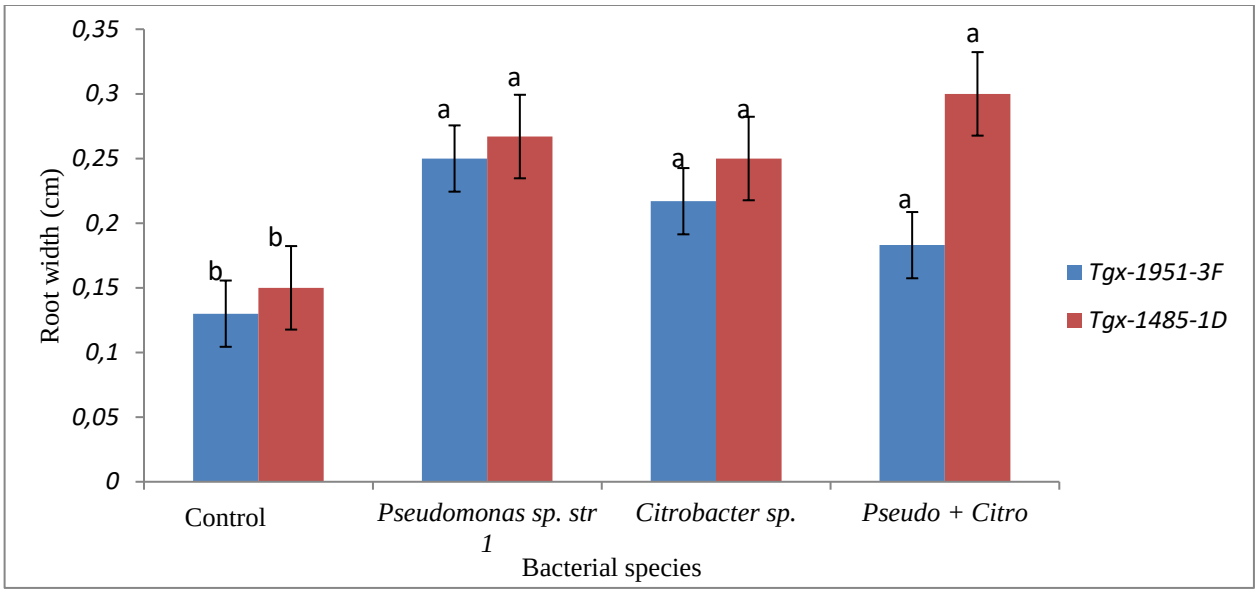

Figure 3: Root width of Tgx-1951-3F and Tgx-1485-1D soybean seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

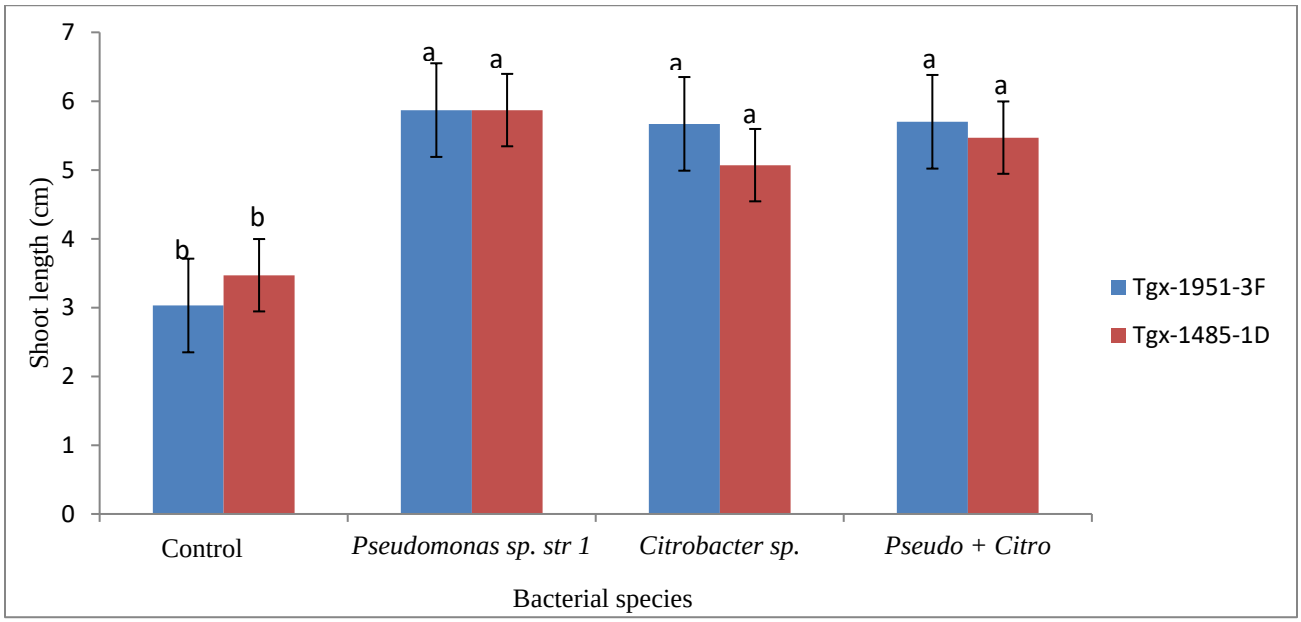

Figure 4: Shoot length of Tgx-1951-3F and Tgx-1485-1D soybean seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., a co-inoculation of *Pseudomonas* sp. Strain 1 and *Citrobacter* sp. and the non-inoculated seedlings(control).

Values represented as mean ± standard deviation

a/b = significant differences ($p < 0.05$)

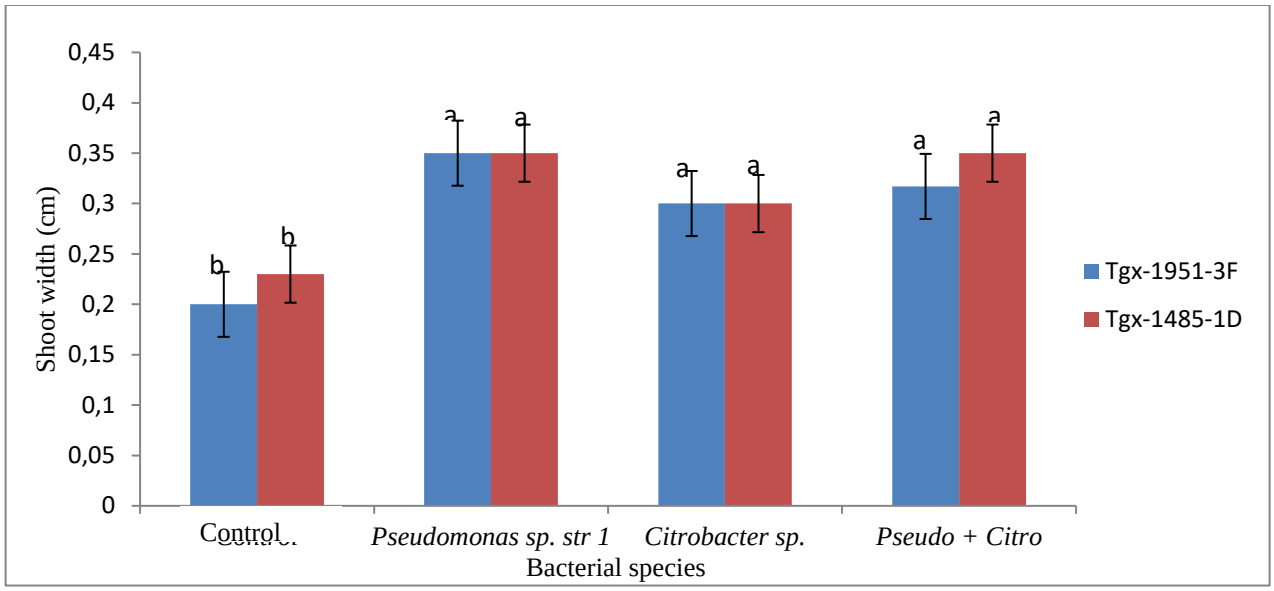

Figure 5: Shoot width of Tgx-1951-3F and Tgx-1485-1D soybean seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

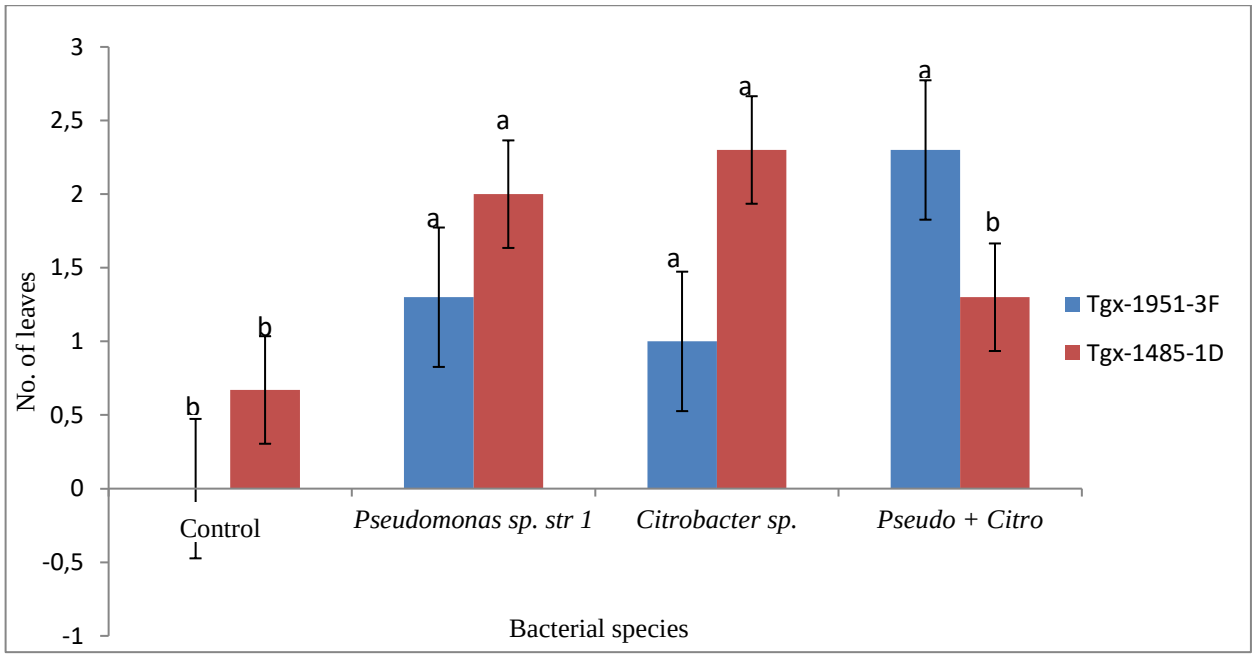

Figure 6: Number of leaves grown by Tgx-1951-3F and Tgx-1485-1D soybean seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

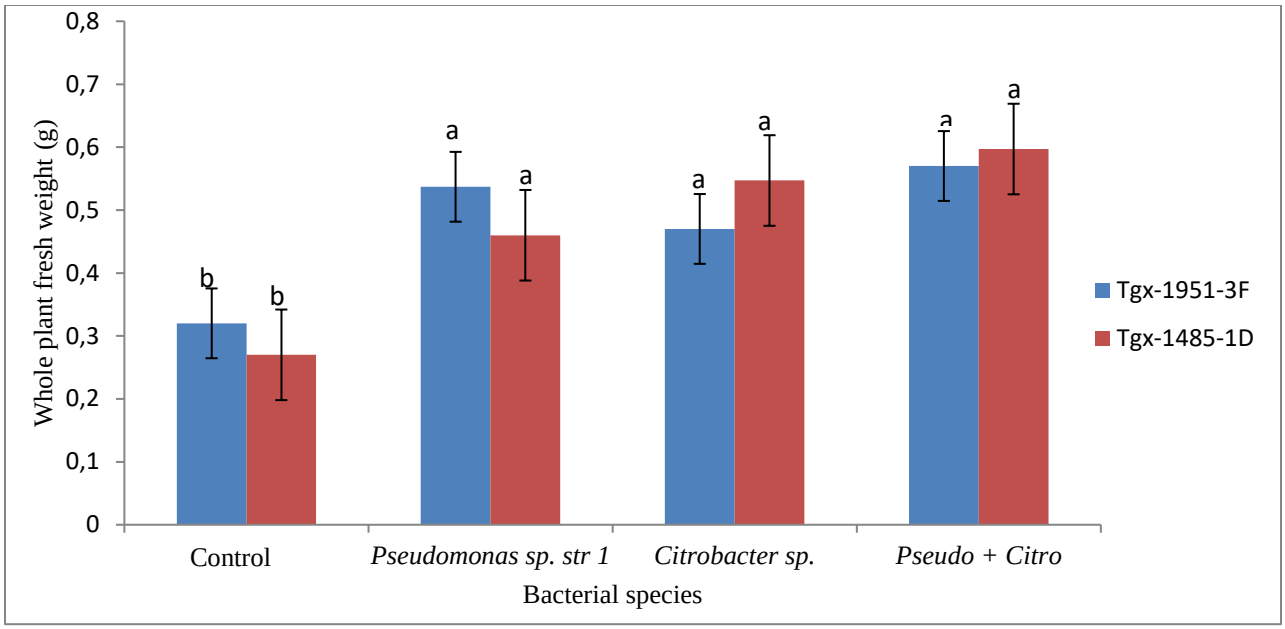

Figure 7: Whole plant fresh weight of Tgx-1951-3F and Tgx-1485-1D soybeans seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

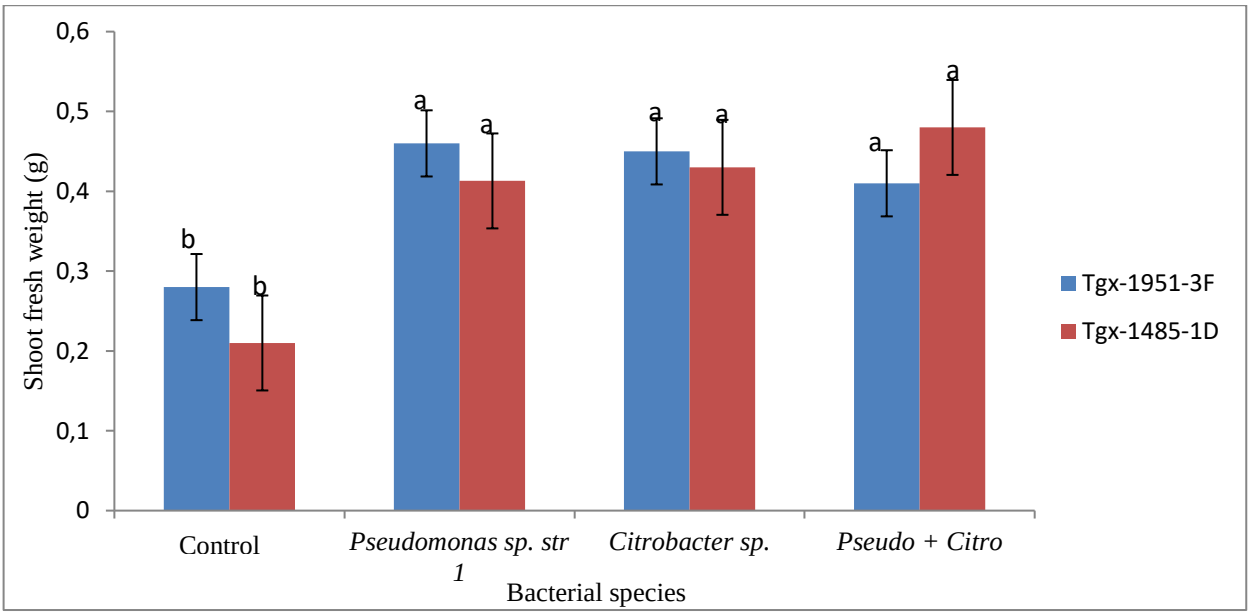

Figure 8: Shoot fresh weight of Tgx-1951-3F and Tgx-1485-1D soybeans seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

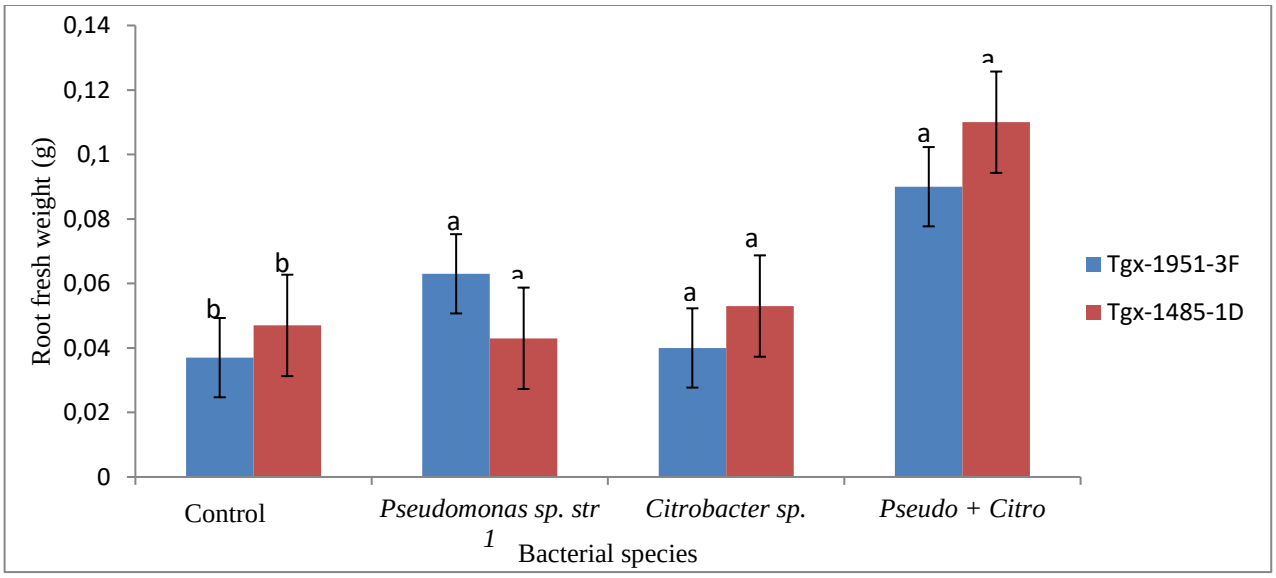

Figure 9: Root fresh weight of Tgx-1951-3F and Tgx-1485-1D soybean seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

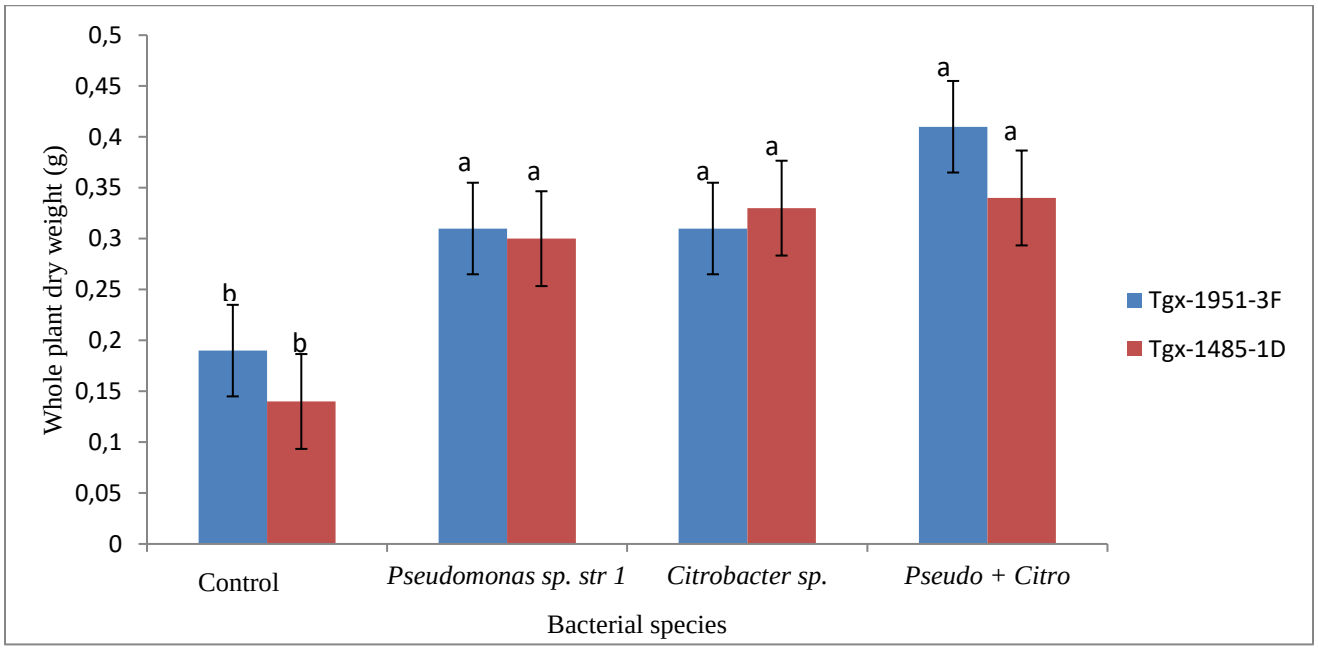

Figure 10: Whole weight dry weight of Tgx-1951-3F and Tgx-1485-1D soybeans seeds inoculated with *Pseudomonas* sp. Strain 1, *Citrobacter* sp., *Pseudomonas* sp. Strain 1 + *Citrobacter* sp. and the non-inoculated seedlings (control).

Legend: Values represent mean ± standard deviation, a/b = significant differences ($p < 0.05$), str represents strain, *pseudo*. represent *Pseudomonas*, *Citro*. represent *Citrobacter*

Plate 1: Positive HCN production

Plate 2: Preparation of bacterial cultures for phosphate solubilization test

Plate 3: Germinated Tgx-1485-1D soybeans seeds at day 7 treated with co-inoculant of *Pseudomonas* sp. strain 1 + *Citrobacter* sp.

Plate 4: Non-inoculated Tgx-1485-1D germinated soybeans seeds

Plate 5: Dried root of germinated soybean seedling

Plate 6: Dried root and shoot of germinated soybean seedling

Plate 7: Germinated Tgx-1951-3F soybeans seed at day 7 treated with co-inoculant of *Pseudomonas* sp. strain 1 + *Citrobacter* sp.

Plate 8: Non-inoculated Tgx-1951-3F soybeans seeds at dav 7

Plate 9: Germinated Tgx-1951-3F soybeans seed at day 7 treated with *Pseudomonas* sp. strain 1

Plate 10: Germinated Tgx-1951-3F soybeans seed at day 7 treated with *Citrobacter* sp.

In the current study, bacterial isolates were isolated, identified, and their qualities that promote plant growth were identified. The isolates were then utilized to inoculate soybean seeds. Three isolates were obtained from tomato rhizospheric soil samples during the isolation and identification process. *Citrobacter* sp., *Yersinia* sp., and *Pseudomonas* sp. strains 1 and 2 were the isolates. Once isolates were found, characteristics that might promote plant development were further searched for. The following four tests were used to promote plant growth: phosphate solubilization, hydrogen cyanide (HCN), indole acetic acid synthesis (IAA), and ammonia production. Depending on the test that was conducted, the bacterial isolates employed in this study had various characteristics that promoted plant development. To be more precise, the amounts of IAA generated varied throughout the bacterial strains. This is consistent with the research that states that the development stage, nutritional availability, and culture conditions may all affect the amount of IAA produced by various bacterial species (Mohite, 2013). Furthermore, compared to bacteria from bulk soil, those from the rhizosphere of plants make IAA more efficient (Sarwar and Kremer, 1995; Loukili *et al.*, 2022). In this study, we observed that *Pseudomonas* sp. strain 1 was more efficient in producing IAA at the concentration of 0.5mg/ml with a concentration of 20.92 ± 0.02, followed by *Citrobacter* sp. with 18.00±0.03 and *Pseudomonas* sp. strain 2 with a concentration of 16.42±0.04. *Yersinia* sp. had the lowest concentration in Indole acetic Acid production.

The ability of rhizospheric bacteria to solubilize phosphate—which is known to always exist in bound forms in the soil—is another tactical tool at their disposal to promote the growth of crops (Putrie et al., 2013).

Strain 1 of *Pseudomonas* sp. was found to be more effective in solubilizing tri-calcium phosphate in Pikovskaya's agar, with a phosphate solubilizing index (PSI) of 2.08±0.03, followed by strain B4 with a PSI of 1.31±0.08, and strain B2 with the least capacity to solubilize phosphate solubilizing potential.

As a result, these bacterial isolates tend to release bound phosphates into the soil so that plants may absorb them.

Many plant diseases appear to be suppressed by hydrogen cyanides (HCN). It has been documented that bacterial isolates decrease root rot by producing a positive connection with HCN production. (Defago *et al*.,1990). Four (4) isolates were tested for qualitative HCN production. *Citrobacter* sp. had the strongest result in terms of producing HCN followed by *Pseudomonas* sp. strain 1 which moderately produced HCN, *Pseudomonas* sp. strain 2, and *Yersinia* sp. showed no positive result, making them unable to produce HCN (Table 4). It has been noted that several factors affect the rate at which HCN is produced. Glycine has been detected in root exudates and has been identified as the immediate precursor to microbial cyanide generation (Voisard *et al.*, 1989, Bakker *et al*.,1989). According to Schippers (1990), microbial HCN may contribute to the establishment of plant resistance even if its function in disease suppression is not thought to be well established. One of the key characteristics connected to the stimulation of plant development is the ammonia generation by Plant development Promoting Rhizobacteria (PGPR). It has been demonstrated that the ammonia generated by PGPR generally provides nitrogen to the host plants, hence promoting biomass and root and shoot elongation. Three (3) isolates in the current investigation produced ammonia in peptone broth (Table 4). A strain of *Pseudomonas* sp. produced the most ammonia.

After analysis of plant growth promoting traits and the production of these PGPR traits, only two of the isolates were used for *in-vitro* enhancement of soybean seed germination. A control without the organism was used to tell if there was any significant change between the treatments. A co-inoculation of the bacterial species was also used as a treatment for soybean seed germination to know whether bacterial synergy would be better or not. The use of *Pseudomonas* sp. strain 1 and *Citrobacter* sp. strains for *in-vitro* enhancement of soybean germination showed that *Pseudomonas* sp. strain 1 and *Citrobacter* sp. strains were able to effectively enhance the germination of soybeans. This result also showed the difference in significance between the inoculated soybean seeds and the non-inoculated soybean seeds (control). The co-inoculation of the bacterial species also enhanced the germination of soybeans and it showed visible difference to non-inoculated soybeans seed (control).

The measurements of root length, root width, shoot width, shoot length, number of leaves, fresh weight, dry weight was taken. The average germinated Tgx-1951-3F soybean seed showed that *Pseudomonas* sp. strain 1 had the highest number of seedling germination (73%), followed by *Citrobacter* sp. (70%) and the co-inoculation (70%), while the control was 30%. In Tgx-1485-1D soybeans, *Pseudomonas* sp. strain 1 also had the highest seedling germination (67%), followed by the co-inoculation (60%), *Citrobacter* sp. showed the lowest seed germination (50%). In the root length of Tgx-1951-3F, all the treatments were significantly different when compared to the non-inoculated seedlings ($p< 0.05$), *Pseudomonas* sp. strain 1 showed the highest root length (5.2±1.085), followed by the co-inoculation (5.015±0.99), then *Citrobacter* sp. (4.83±0.9). In comparison, Tgx-1485-1D seedling germination also showed significantly different results in root length when compared with the non-inoculated seedlings. The only difference between Tgx-1951-3F and Tgx-144-1D in root length was that Tgx-1485-1D had the highest root length in the co-inoculation treatment (4.47±1.185). In the root width measurement of Tgx-1951-3F, *Pseudomonas* sp. strain 1 also showed the highest growth (0.25±0.059), followed by

Citrobacter sp. (0.22±0.045) and the co-inoculation (0.185±0.025), this result showed that the soybeans seeds were enhanced more when inoculated with these bacterial species. In comparison, Tgx-1485-1D, also showed that *Pseudomonas* sp. strain 1 treatment had the highest root length (0.267±0.115) while the co-inoculation treatment had the least root width (0.23±0.135). In the shoot length of Tgx-1951-3F and Tgx- 1485-1D, *Pseudomonas* sp. strain 1 showed highest growth (5.87±1.42, 5.87±1.205), the shoot length of the co-inoculation of both seedling varieties was also similar, in Tgx-1951-3F, shoot length of *Citrobacter* sp. treatment was higher than that of Tgx-1485-1D, but showed a huge difference when compared with the control. In the measurement of number of leaves, the co-inoculation had the highest measurement (2.33±1.165) for variety Tgx-1951-3F while in variety Tgx-1485-1D, the *Citrobacter* sp. strain treatment had the highest number (2.33±0.045) and there was no growth of leaves in the control.

The whole plant fresh weight of Tgx-1951-3F and Tgx-1485-1D had their co-inoculated seedling weighing more than other inoculated seedlings (0.57±0.124, 0.59±0.16) while in Tgx-1951-3F *Pseudomonas* sp. strain 1 treatment weighed more than Tgx-1485-1D variety. The dry weight of both varieties weighed more in the co-inoculated treatment (0.407±0.11, 0.34±0.09). They weighed more than the control and showed greater enhancement.

Conclusion

New practices must be harnessed and used to reduce food insecurity in the country. Microbiology has played a great part in reducing food insecurity and is still playing such roles to date. Plant growth-promoting rhizobacteria (PGPRs) are useful in augmenting plant development due to the characteristics that make them up. By increasing soil nutrient absorption of nitrogen, potassium, and phosphorus under both normal and drought-stressed circumstances, PGPR treatment has a positive impact on soybean plant growth and nodulation. As a result, the potential PGPR capacity of these bacterial strains may be further developed for biotechnological use in the field, particularly in the nation's semiarid and arid regions, boosting food production.

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