Genetic Characterisation and Symbiotic Efficiency of Rhizobia Nodulating Different Landraces of Bambara Groundnut (*Vigna subterranea* L. Verdc)

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Abstract: The identification of native rhizobia capable of inducing effective nodulation in legumes is a prerequisite for selection of strains for bioinoculation. The study aimed to characterize and evaluate the symbiotic efficiency of native rhizobia capable of effectively nodulating Bambara groundnut (*Vigna subterranean*). Greenhouse experiment was conducted in a completely randomized design with five accessions of Bambara groundnut and five replications. The rhizobia strains were isolated from the root nodules and subjected to nodulation test on the host plants. The rhizobial isolates were characterized by morpho-cultural characteristics and 16S rRNA gene sequencing technique. The symbiotic efficiency of the isolates on Bambara groundnut was also evaluated as data on dry shoot weights, nodule number, fresh and dry weights were collected. Of the thirty five rhizobial isolates obtained, only 15 (42.9%) successfully induced effective nodulation in Bambara groundnut plants and the 16S rRNA gene sequencing identified them as strains of *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Rhizobium*. The results of the symbiotic efficiency of rhizobia showed significant variations in the symbiotic capacities of the strains. The nodule number, fresh and dry weights ranged from 13.67±0.88 to 57.33±2.03 per plant, 0.10±0.01 to 0.33±0.03 g/plant and 8.33±0.67 to 40.00±1.73 mg/plant, respectively. The strains enhanced the dry shoot weights by 36.7% to 121.8% over non-inoculated control. The study therefore revealed the potential of native strains of rhizobia in nodulating Bambara groundnuts and these strains could be considered as potential inoculant strains for sustainable production of Bambara groundnut in Nigeria.
Key word: Rhizobia, *Vigna subterranean*, nodulation, symbiotic efficiency, inoculant

INTRODUCTION

The use of microbial inoculants for the enhancement of sustainable agricultural production is becoming a more widely accepted practice in intensive agriculture in many parts of the world (Majeed *et al*., 2015). The economic impacts of the use of inorganic fertilizers and the negative impacts to the environment became a global concern (Osoro *et al*., 2014).

Bambara groundnut (*Vigna subterranea* L. Verdc) is a crop commonly cultivated in sub-Saharan Africa by subsistence farmers (Ibny *et al*., 2019). The crop is the third most important grain legume in Africa after cowpea and groundnut (Puozaa *et al*., 2017). It is one of the under-utilized African legumes grown for its grains and it is usually cultivated either as a monoculture or in mixed culture/rotation with cereals. Nutritionally, the grain of this crop serves as a balanced meal due to its richness in protein, carbohydrate, fat and fibre (Onyango *et al*., 2015; Puozaa *et al*., 2017; Ibny *et al*., 2019). The leaves are also high in nitrogen and potassium which therefore could serve as an excellent protein-rich feeds for animals (Ibny *et al*., 2019). Aside from the nutritional, it also has several agronomic advantages like drought tolerance and ability to produce high yields in soils that are low in nutrients (Guei *et al*., 2019).

Like other legumes, Bambara groundnut also nodulates profusely and fixes atmospheric nitrogen, thereby involving in replenishing soil nitrogen. It is capable of fixing atmospheric nitrogen through symbiotic relationship with rhizobia (Guei *et al*., 2019), which results in satisfying the nitrogen demand of the host plant and thus, directly promoting the growth. However, the native rhizobia may exhibit high levels of N_2 fixation and plant growth promotion when compared to the introduced commercial strains (Onyango *et al*., 2015). Although, the genetic, economic and nutritional potentials of Bambara groundnut have been recognized, the crop has not received adequate research attention in terms of legume-microbes interactions when compared to legumes like groundnut,

cowpea and soybean (Hillocks *et al*., 2012; Ngo *et al*., 2015). Therefore, the study was conducted to characterize and evaluate the symbiotic efficiency of native rhizobia capable of effectively nodulating Bambara groundnut, which is a necessary step towards improving its production in Nigeria.

MATERIALS AND METHODS

Experimental site: Pot experiment was carried out in the greenhouse of the Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria. The study location lies within the rain forest agro-ecological zone of South-west Nigeria (Latitude 7^{o}N, Longitude 3.5^{o}E in Odeda Local government of Ogun-state). The planting soil was obtained from the FADAMA farm, Federal University of Agriculture, Abeokuta.

Source of Bambara groundnuts: Seeds of five Bambara groundnut accessions (TVSU465, TVSU729, TVSU833, TVSU1626 and TVSU1939) with similar growth habitats but different seedcoat colours were sourced from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State (Figure 1).

Experimental design and planting of Bambara groundnuts: The experiment was conducted in a completely randomized design with five accessions and five replicates per accession. Each replicate consisted of a single pot with one plant per pot. The seeds were surface-sterilized with 95% ethanol for one minute, followed by 2% sodium hypochlorite solution for one minute, rinsed five times in sterile distilled water and air dried at 25±2^{o}C. Two seeds were sown in each pot and thinned to one plant per pot at seven days after sowing. All pots were placed in a greenhouse at 25 to 30°C and 12 h photoperiod. The plants were watered twice a day (morning and evening) without fertilizer application.

Isolation and authentication of root nodule bacteria from Bambara groundnuts: At 45 days after planting, Bambara groundnut plants were carefully uprooted and the soil was washed off from the roots without detaching the nodules. Pink nodules were then collected from the roots of each plant and used for isolation of root nodule bacteria. Soil samples were also collected from the rhizosphere (0 - 10 cm depth) of the five plants in each accession and bulked into a composite sample for isolation of rhizobacteria.

The isolation of nodule bacteria from the root nodules of Bambara groundnuts was performed using the methods described by Onyango *et al*. (2015) and Koskey *et al*. (2018) with slight modifications. Briefly, the nodule samples were washed, surface-sterilized in 1% sodium hypochlorite solution for 3 minutes, rinsed in five changes of sterile distilled water and air-dried. Then, the nodules were crushed and the resulting milky suspension was inoculated (in triplicates) on Yeast extract mannitol agar (YEMA) plates containing yeast extract (1.0 gL^{-1}), mannitol (10.0 gL^{-1}), K_2PO_4 (0.5 gL^{-1}), $MgSO_4$ (0.2 gL^{-1}), NaCl (0.1 gL^{-1}), congo red (25 mgL^{-1}) and agar (20.0 gL^{-1}), adjusted to pH 6.8 with 0.1M NaOH. The plates were then incubated in the dark at 28 ± 2^{o}C for 5 days. After incubation, the colonies were sub-cultured on fresh YEMA plates to obtain the pure cultures of root nodule bacteria.

The potential of the root nodule bacterial isolates to nodulate their respective host plants was evaluated by nodulation test described by Ngo *et al*. (2015) and Zou *et al*. (2016) with little modifications. Seeds of Bambara groundnuts were surface-sterilized with 95% ethanol for five minutes, followed by 2% sodium hypochlorite solution for two minutes, rinsed five times in sterile distilled water and air-dried. The seeds were then scarified with concentrated H_2SO_4, to soften the thick and hard seed coat, rinsed six times with sterile distilled water and pre-germinated on moistened sterile tissue papers in petri-dishes. The pre-germinated seeds were sown into pots containing sterilized soil. Seven days after planting, the

pots were inoculated with 1.0 ml of 1×10^8 cfu/ml of YEM broth culture of each isolate with each treatment replicated three times. Pots inoculated with 1.0 ml of sterile YEM broth served as control. Plants were watered regularly with N-free nutrient solution. The plants were then harvested six weeks after planting and their roots were assessed for the presence of effective nodules. The isolates that induced effective nodule formation on the test host plants were considered as true rhizobia. The pure cultures of true rhizobia isolates were maintained on YEMA slants and preserved in the refrigerator at 4ºC.

Isolation of bacterial strains from rhizosphere of Bambara groundnuts

Isolation of rhizobacteria was performed by adopting the methods described by Dinesh *et al*. (2015) and Islam *et al*. (2016) with little modifications. Ten grams (10.0 g) of each soil sample was aseptically suspended in 90.0 ml of sterile distilled water and shaken vigorously on a rotary shaker for about 15 minutes. The suspension was then allowed to settle for about 10 minutes and serially diluted. Then, 1.0 ml each of 10^{-3} and 10^{-5} aliquot was inoculated on nutrient agar (in triplicates) using pour plate method. The plates were incubated at 28 ± 2ºC for 48 hours. Pure bacterial isolates were obtained by sub-culturing on fresh nutrient agar plates. The pure cultures of bacterial isolates were maintained on nutrient agar slants and preserved at 4°C.

Phenotypic characterization of bacterial isolates

Pure bacterial isolates were characterized phenotypically on the basis of their cultural, morphological and biochemical methods. The true rhizobia isolates were further classified as slow-growers (> 5 days), intermediate growers (4 - 5 days) and fast-growers (< 4 days) by streaking them on YEMA plates and incubated at 28 ± 2ºC for 15 days.

Molecular characterization of rhizobial isolates

The molecular characterization of effective rhizobia isolates was performed by 16S rRNA gene sequencing method. The extraction and purification of total genomic DNA was done using Zymo (ZR) fungal/bacterial genomic DNA extraction kit (Zymo Research, USA) following the manufacturer's instructions.

The PCR amplification of the 16S rRNA genes of the isolates was conducted with the primer pair of 27F (5'- AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-ACG GCT ACC TTG TTA CGA CTT-3'). Each of the reactions was carried out in a total volume of 20.0 µl consisting of 1.5 µl of template DNA, 10.0 µl of 2× PCR master mix (Norgen Biotek Corporation, Canada), 1.0 µl of forward primer (2.5µM), 1.0 µl of reverse primer (2.5µM) and 6.5 µl of nuclease-free water. The amplification conditions were as follows: initial denaturation of 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 sec, extension of 72 °C for 5 min, final extension of 72 °C for 10 min and hold at 4°C. A 1.0% (w/v) agarose gel electrophoresis in 1x TAE buffer at 100V for 1 hour was used to separate the amplified PCR products in the presence of a 1Kb PCR ladder. The amplicons were then purified and sequenced in an automated gene sequencer (ABI 3730XL) using 27F and 1492R primers. Gene sequences were compared with GenBank database at National Centre for Biotechnology Information *(http://www.ncbi.nlm.nih.gov.)* using BLASTn search tool to identify the isolates. A Neighbour-joining dendrogram showing the phylogenetic relatedness of the rhizobia isolates was constructed using MEGA 5.2 software.

Symbiotic efficiency of native rhizobial isolates on Bambara groundnut: The symbiotic potential of true rhizobia isolates was evaluated on Bambara groundnut TVSU1626 using the methods described by Osei *et al*. (2018) and Ibny *et al*. (2019) with little modifications. Seeds of Bambara groundnut TVSU1626 were surface-sterilized, scarified and pre-germinated as previously described. The pre-germinated seeds were then planted in sterilized plastic pots filled with autoclaved sand. Seven days

after planting, the seedlings were inoculated with 1.0 ml of 10^8 cells per ml of each rhizobia isolate, with each treatment replicated three times. Un-inoculated plants served as control. Plants were watered weekly with N-free nutrient solution. The plants were then harvested 45 days after sowing and their roots were assessed for the presence of nodules. The soil adhering to the roots was carefully loosened, ensuring that the roots were not disturbed. The roots were gently washed under running water to remove the soil particles, and air-dried. The nodules were removed, counted and weighed to determine the nodule number and fresh nodule weight per plant. The nodules and shoots were oven-dried at 60°C for 3 days and weighed to determine the dry nodule weight and shoot dry weight respectively.

Statistical analysis of data obtained: Data collected were analyzed by one-way analysis of variance. Means were separated using Duncan's Multiple Range Test at $p \leq 5\%$.

RESULTS

The populations of bacteria in the rhizosphere of five accessions of Bambara groundnuts are shown in Table 1. This indicates that the bacterial population of Bambara groundnut TVSU1626 was significantly ($p \leq 0.05$) higher than that of other accessions. The mean rhizobacterial population was found to be highest (71.00×10^5 cfu/g) in the TVSU1626 and the least population (34.0×10^5 cfu/g) was found in the TSVU833.

Figure 1: Seeds of Bambara groundnut accessions used. A: TVSU465; B: TVSU729; C: TVSU833; D: TVSU1626 and E: TVSU1939

Table 1: Bacterial populations in the rhizosphere of different accessions of Bambara groundnuts

Accession Number	Total rhizobacterial counts ($\times10^5$cfu/g ± S.E.)
TVSU 465	54.67 ± 3.71^{b}
TVSU 729	37.00 ± 5.86^{c}
TVSU 833	34.00 ± 1.00^{c}
TVSU 1626	71.00 ± 4.04^{a}
TVSU 1939	45.00 ± 2.08^{bc}

Key: Values are means of three plates per sample ± standard errors. Means with the different letters along the column are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

A total of 35 bacterial strains were obtained from the root nodules of five accessions of Bambara groundnuts (Table 2). Out of these 35 isolates, only 15 isolates (42.9%) were able to induce effective nodules on their host plants and these isolates were considered as true rhizobia. Five isolates (14.2%) formed non-effective nodules while the remaining 15 isolates failed to nodulate their host plants. Amongst these true rhizobia isolates, two isolates (BG03 and BG06) were obtained from Bambara groundnut TVSU465, three isolates (BG10, BG13 and BG14) from accession TVSU729, three isolates (BG15, BG18 and BG20) were obtained from accession TVSU833, five isolates (BG22, BG25, BG26, BG27 and BG28) were recovered from TVSU1626 while two isolates (BG31 and BG34) were found in Bambara groundnut TVSU1939.

All these effective root nodule bacterial isolates were Gram negative and rod-shaped cells (Table 3). About 45% of the root nodule bacteria were fast growers appearing on YEMA plates within 2 to 4 days of incubation while the remaining isolates were either intermediate growers or slow growers. The molecular identification of the true rhizobia isolates showed 92% to 98% similarities to strains of *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium* and *Sinorhizobium* species, with *Bradyrhizobium* species dominating (Table 4). In addition, Figure 2 shows a Neighbor joining dendrogram of effective root nodule bacteria associated with Bambara groundnuts with isolates BG03, BG06, BG13, BG26, BG28 and BG31 clustering with species of *Bradyrhizobium*, indicating their close relatedness. Isolates BG10, BG15, BG18, BG22 and BG25 also clustered with species of *Rhizobium*; isolate BG27 clustered with *Mesorhizobium thiogangeticum* while isolates BG20 and BG34 clustered with *Sinorhizobium saheli*. Similarly, a total of sixty five (65) bacterial strains belonging to 10 bacterial genera were isolated from the rhizosphere of five accessions of Bambara groundnuts. *Pseudomonas* was recorded as the most frequently occurring genus with four species (*Pseudomonas aeruginosa, P. alcaligenes*, *P. syringae* and *P. fluorescens*), followed by *Bacillus* with three species (*B. subtilis, B. licheniformis* and *B. megaterium*) and *Kurthia* with two species (*K. gibsonii* and *K. zopfii*). Other rhizobacterial isolates were strains of *Alcaligenes faecalis*, *Acinetobacter* sp, *Enterobacter aerogenes, Klebsiella pneumoniae, Escherichia coli* and *Micrococcus* sp. The occurrence of the bacterial isolates in the rhizosphere of Bambara groundnuts is shown in Figure 3.

Table 2: Cultural characteristics of root nodule bacteria isolated from different accessions of Bambara groundnuts

Isolate	Source	Size	Colour	Elevation	Margin	Opacity	Texture	Growth pattern
BG 01	TVSU465	Medium	Milky white	Flat	Entire	Translucent	Gummy	Intermediate
BG 02	TVSU465	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast
BG 03	TVSU465	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 04	TVSU465	Small	Cream white	Convex	Smooth	Opaque	Mucoid	Slow
BG 05	TVSU465	Medium	Milky white	Flat	Entire	Translucent	Gummy	Intermediate
BG 06	TVSU465	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 07	TVSU465	Small	Yelloe	Convex	Smooth	Opaque	Mucoid	Slow
BG 08	TVSU729	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast
BG 09	TVSU729	Large	Cream white	Convex	Smooth	Opaque	Mucoid	Slow
BG 10	TVSU729	Small	Milky white	Raised	Entire	Opaque	Gummy	Fast
BG 11	TVSU729	Small	Yellow	Convex	Smooth	Opaque	Mucoid	Slow
BG 12	TVSU729	Small	Cream white	Convex	Smooth	Opaque	Mucoid	Slow
BG 13	TVSU729	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 14	TVSU729	Large	Yellow	Convex	Entire	Transparent	Mucoid	Fast
BG 15	TVSU833	Small	Milky white	Raised	Entire	Opaque	Gummy	Fast
BG 16	TVSU833	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast
BG 17	TVSU833	Medium	Milky white	Flat	Entire	Translucent	Gummy	Intermediate
BG 18	TVSU833	Small	Milky white	Raised	Entire	Opaque	Gummy	Fast
BG 19	TVSU833	Small	Cream white	Raised	Smooth	Opaque	Mucoid	Slow
BG 20	TVSU833	Medium	Cream white	Convex	Entire	Translucent	Gummy	Fast
BG 21	TVSU833	Small	Cream white	Convex	Smooth	Opaque	Mucoid	Slow
BG 22	TVSU1626	Small	Milky white	Raised	Entire	Opaque	Gummy	Fast
BG 23	TVSU1626	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast
BG 24	TVSU1626	Medium	Milky white	Flat	Entire	Translucent	Gummy	Intermediate
BG 25	TVSU1626	Small	Milky white	Raised	Entire	Opaque	Gummy	Fast
BG 26	TVSU1626	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 27	TVSU1626	Large	Yellow	Convex	Entire	Transparent	Mucoid	Fast
BG 28	TVSU1626	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 29	TVSU1939	Small	Yellow	Flat	Entire	Translucent	Gummy	Fast
BG 30	TVSU1939	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast
BG 31	TVSU1939	Medium	Cream white	Flat	Smooth	Translucent	Gummy	Slow
BG 32	TVSU1939	Large	Cream white	Flat	Smooth	Opaque	Mucoid	Slow
BG 33	TVSU1939	Small	Cream white	Convex	Smooth	Opaque	Mucoid	Slow
BG 34	TVSU1939	Small	Milky white	Convex	Entire	Opaque	Gummy	Fast
BG 35	TVSU1939	Medium	Milky white	Flat	Entire	Translucent	Gummy	Fast

Table 3: Morphological and biochemical characteristics of effective root nodule bacteria isolated from Bambara groundnuts

Isolates	Gram	Cell shape	Glucose	Sucrose	Lactose	H_2S	Catalase	Citrate	Starch hydrolysis	Urease	Oxidase	Motility	Suspected organisms
BG03	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG06	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG10	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.
BG13	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG14	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG15	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.
BG18	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.
BG20	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG22	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.
BG25	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.
BG26	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG27	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG28	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG31	−	Rod	−	+	+	+	+	+	+	+	+	+	*Bradyrhizobium* spp.
BG34	−	Rod	+	+	+	+	+	+	+	+	+	+	*Rhizobium* spp.

Key: +: Positive reaction −: Negative reaction

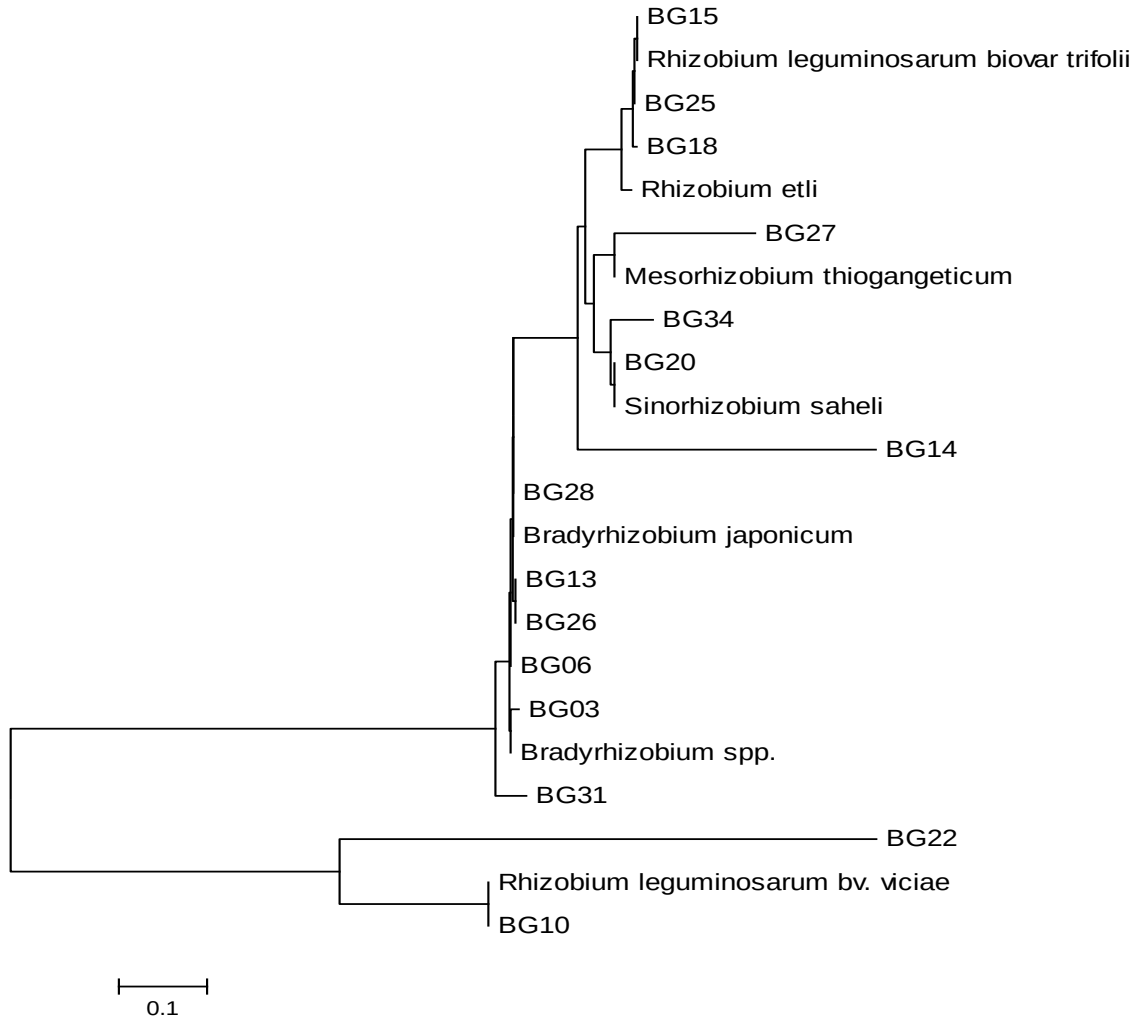

Figure 2: A Neighbor Joining Dendrogram of effective root nodule bacteria associated with Bambara groundnuts

Table 4: Phenotypic and molecular identification of effective root nodule bacteria isolated from different accessions of Bambara groundnuts

Isolate	Accession	Phenotypic identification	Molecular identification	% Similarity at NCBI
BG03	TVSU465	*Bradyrhizobium* sp.	*Bradyrhizobium* sp. strain B918	95.0
BG06	TVSU465	*Bradyrhizobium* sp.	*Bradyrhizobium japonicum*	98.0
BG10	TVSU729	*Rhizobium* sp.	*Rhizobium leguminosarum* biovar *viciae*	97.0
BG13	TVSU729	*Bradyrhizobium* sp.	*Bradyrhizobium japonicum*	96.0
BG14	TVSU729	*Bradyrhizobium* sp.	*Mesorhizobium thiogangeticum*	92.0
BG15	TVSU833	*Rhizobium* sp.	*Rhizobium leguminosarum* biovar *trifolii*	95.0
BG18	TVSU833	*Rhizobium* sp.	*Rhizobium etli*	93.0
BG20	TVSU833	*Bradyrhizobium* sp.	*Sinorhizobium saheli* strain RC10	95.0
BG22	TVSU1626	*Rhizobium* sp.	*Rhizobium leguminosarum*	96.0
BG25	TVSU1626	*Rhizobium* sp.	*Rhizobium leguminosarum* biovar *trifolii*	95.0
BG26	TVSU1626	*Bradyrhizobium* sp.	*Bradyrhizobium* sp. strain B918	98.0
BG27	TVSU1626	*Bradyrhizobium* sp.	*Mesorhizobium* sp. strain SW3	94.0
BG28	TVSU1626	*Bradyrhizobium* sp.	*Bradyrhizobium japonicum*	96.0
BG31	TVSU1939	*Bradyrhizobium* sp.	*Bradyrhizobium japonicum*	98.0
BG34	TVSU1939	*Rhizobium* sp.	*Sinorhizobium saheli* strain RC10	94.0

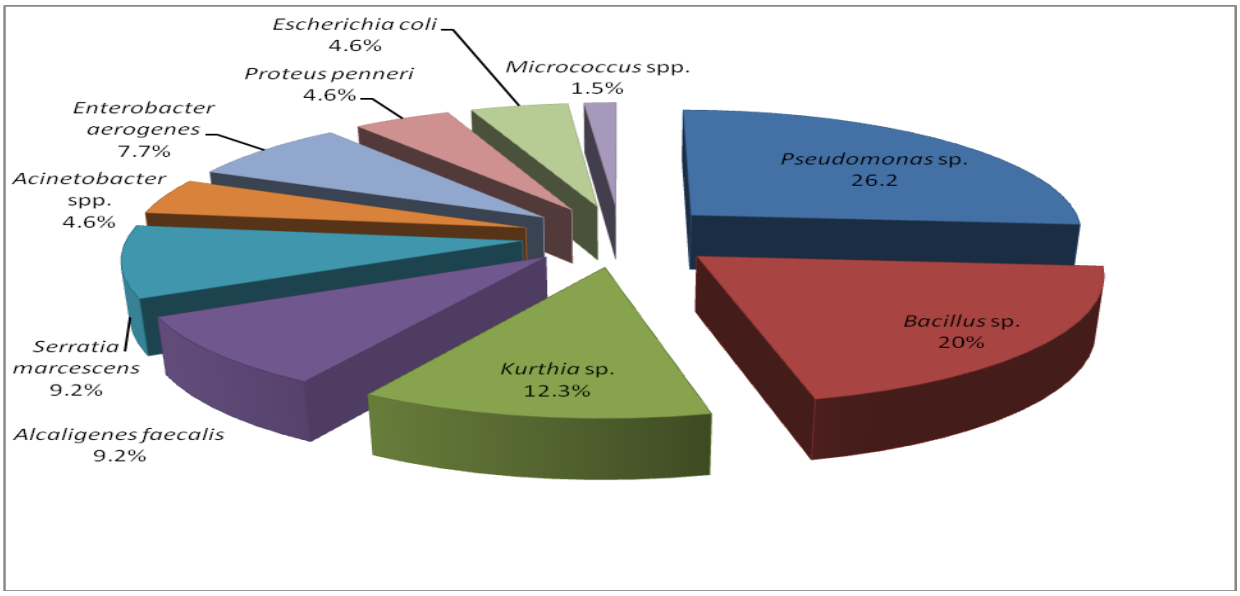

Figure 3: Percentage occurrence of bacteria in the rhizosphere of Bambara groundnuts

The findings of the symbiotic potential of the true rhizobia isolates showed significant differences ($p \leq 0.05$) in the nodule numbers, dry shoot weights, fresh and dry nodule weights of Bambara groundnut (Table 5). All the fifteen screened rhizobia isolates induced effective nodule formation in test Bambara groundnut with mean nodule numbers ranging from 13.67±0.88 to 57.33±2.03 per plant. The strains of *Bradyrhizobium* species generally induced significantly ($p \leq 0.05$) higher nodules than the strains of *Rhizobium* species. The non-inoculated plants did not form any nodule (Table 5). The fresh and dry nodule weights were significantly higher in plants inoculated with *Bradyrhizobium* species than those inoculated with *Rhizobium* species.

The mean fresh nodule weights of the inoculated plants ranged from 0.10±0.01 to 0.33±0.03 g/plant while the dry nodule weights ranged from 8.33±0.67 to 40.00±1.73 mg/plant (Table 5). Compared to the non-inoculated control, the rhizobia isolates significantly ($p \leq 0.05$) increased the dry shoot weights of the Bambara groundnut seedlings. There were significant differences ($p \leq 0.05$) between the mean dry shoot weights of inoculated plants and non-inoculated plants (Table 5). The rhizobia strains enhanced the dry shoot weights by 36.7% to 121.8% over non-inoculated

control. It was however observed that the *Bradyrhizobium* species significantly performed better than other rhizobia strains. Therefore, the symbiotic effectiveness of the rhizobia isolates in Bambara groundnut varied significantly.

Table 5: Symbiotic efficiency of rhizobial strains with Bambara groundnut

Rhizobial isolate	Shoot dry weight (g/plant ± S.E)	Nodule number/ plant ± S.E.	Fresh nodule weight (g/plant ± S.E.)	Dry nodule weight (mg/plant ± S.E.)
Bradyrhizobium sp. BG03	1.17 ± 0.09^{a}	47.00 ± 2.00^{b}	0.22 ± 0.03^{bcde}	21.33 ± 0.88^{c}
B. japonicum BG06	1.08 ± 0.06^{a}	35.00 ± 2.08^{c}	0.18 ± 0.01^{defg}	26.00 ± 3.46^{c}
R. leguminosarum BG10	0.75 ± 0.02^{b}	13.67 ± 0.88^{e}	0.10 ± 0.01^{g}	8.33 ± 0.67^{d}
B. japonicum BG13	1.00 ± 0.10^{a}	32.67 ± 1.67^{c}	0.20 ± 0.02^{cdef}	21.33 ± 1.67^{c}
Mesorhizobium sp. BG14	0.91 ± 0.04^{ab}	33.67 ± 2.33^{c}	0.22 ± 0.01^{bcde}	22.33 ± 2.85^{c}
R. leguminosarum. BG15	0.89 ± 0.20^{b}	14.33 ± 1.86^{e}	0.17 ± 0.03^{efg}	12.33 ± 1.86^{d}
R. etli BG18	0.77 ± 0.05^{b}	17.33 ± 1.86^{de}	0.11 ± 0.01^{g}	12.00 ± 1.53^{d}
Sinorhizobium saheli BG20	1.06 ± 0.05^{a}	48.33 ± 2.91^{b}	0.22 ± 0.01^{bcde}	28.00 ± 2.89^{bc}
R. leguminosarum BG22	0.76 ± 0.20^{b}	22.00 ± 2.08^{d}	0.16 ± 0.01^{efg}	14.33 ± 0.88^{d}
R. leguminosarum BG25	0.85 ± 0.14^{b}	29.33 ± 0.88^{cd}	0.14 ± 0.01^{fg}	14.33 ± 1.67^{d}
Bradyrhizobium spp. BG26	1.02 ± 0.17^{a}	54.00 ± 4.93^{ab}	0.33 ± 0.03^{a}	40.00 ± 1.73^{a}
Mesorhizobium spp. BG27	1.05 ± 0.13^{a}	51.67 ± 2.33^{ab}	0.29 ± 0.02^{ab}	38.33 ± 2.33^{a}
B. japonicum BG28	1.22 ± 0.10^{a}	57.33 ± 2.03^{a}	0.27 ± 0.04^{abc}	35.33 ± 1.45^{a}
B. japonicum BG31	0.96 ± 0.06^{ab}	47.67 ± 2.19^{b}	0.25 ± 0.02^{bcd}	33.67 ± 3.48^{ab}
S. saheli BG34	0.82 ± 0.13^{b}	18.67 ± 0.33^{de}	0.15 ± 0.03^{efg}	13.67 ± 2.73^{d}
Non-inoculated control	0.55 ± 0.11^{c}	0.00 ± 0.00^{f}	0.00 ± 0.00^{h}	0.00 ± 0.00^{e}

Key: Values are means of three plants per isolate ± standard errors. Means with the different letters along the columns are significantly different ($p \leq 0.05$) using Duncan's Multiple Range Test.

DISCUSSION

The nodulation of legumes with highly effective strains of rhizobia has been recognized as an essential and feasible means of promoting biological nitrogen fixation with subsequent increase in grain yields (Osei *et al*., 2018). However, lack of exotic and local strains of rhizobia as inoculants could contribute to the decrease in nodulation, growth and productivity of bambara groundnuts in many African countries. In the present study, the total bacterial populations recorded in the rhizosphere soil samples ranged from 34.00 $\times 10^5$cfu/g to 71.00 $\times 10^5$cfu/g. These populations are much lower than the total bacterial populations obtained for the rhizosphere of some plants analyzed by Oloyede *et al*. (2017). The variations in bacterial populations of the seedlings could be attributed to the influence of root exudates' chemical composition on the microorganisms as well as the plant genotype. Also in this study, 35 bacterial strains were isolated from the root nodules of five accessions of Bambara groundnuts, but only 15 isolates (42.9%) were considered as true rhizobia, 14.2% formed non-effective nodules while 42.9% isolates were non-nodulating bacteria. Previous study of Ngo *et al*. (2015) reported 66.7% true rhizobia from Bambara groundnuts while Osei *et al.* (2018) obtained 20% true rhizobia from groundnuts. The effective nodules were observed to be pink indicating the presence of high concentration of leghaemoglobin. The presence of non-nodulating bacteria in root nodules had also been reported in other legumes like soybean (Li *et al*., 2008) and cowpea (Chidebe *et al*., 2018). The results of morphology, biochemical characterizations and 16S rRNA gene sequencing further confirmed the isolates to be *Bradyrhizobium, Sinorhizobium, Mesorhizobium* and *Rhizobium* species. These results are consistent with numerous studies that indicate *Bradyrhizobium* and *Rhizobium* species to induce nodulation in Bambara groundnuts (Ngo *et al*., 2015; Onyango *et al*., 2015, Puozaa *et al*., 2017; Guei *et al*., 2019; Ibny *et al*., 2019). The effective nodulation of Bambara groundnuts by these bacteria could be mediated by *nod* genes which are naturally present in the rhizobia (Zou *et al*., 2016).

The diversity and composition of the rhizobacteria of a plant could be influenced by the plant species, soil type and soil management practices as well as the interactions among the microorganisms in the rhizosphere (Ahmed *et al*., 2014). Also in this study, 65 bacterial strains were isolated from the rhizosphere of Bambara groundnut seedlings and majority of which belonged to the genus *Pseudomonas*, followed by *Bacillus*. Others belonged to the genera *Kurthia*, *Alcaligenes*, *Acinetobacter, Enterobacter, Klebsiella, Proteus, Escherichia* and *Micrococcus*. The study therefore revealed that the rhizosphere of plants serves as natural reservoirs of several groups of bacteria with diversified characteristics, indicating the importance and richness of this region as a source of plant – microbe interactions. These findings are in agreement with the previous studies that have isolated the species of *Pseudomonas, Bacillus, Alcaligenes, Acinetobacter, Enterobacter, Micrococcus and Kurthia* from the rhizosphere of most plants (Dinesh *et al*., 2015; Islam *et al*., 2016; Mohammed *et al*., 2020).

The nodule numbers of Bambara groundnut inoculated with true rhizobia isolates ranged from 13.67 to 57.33 per plant. Earlier report by Onyango *et al*. (2015) also found that *Bradyrhizobium, Burkholderia* and *Rhizobium* species produced highly effective nodules in Bambara groundnuts with nodule number above 20 per plant. The results further revealed that the native rhizobia isolates exhibited the potential to significantly increase the shoot biomass of Bambara groundnuts. These findings agreed with the previous studies that demonstrated native rhizobia strains being effective in promoting growth and yields of Bambara groundnuts and other legumes (Osei *et al*., 2018; Guei *et al*., 2019; Ibny *et al*., 2019). The variations in the fresh and dry nodule weights could be attributed to the variations observed in the number of nodules. Variations in the symbiotic capacities of several rhizobia strains have also been reported in several studies (Marra *et al*., 2012; Osei *et al*., 2018).

CONCLUSION

The study reveals that *Bradyrhizobium* species are mostly responsible for the nodulation of Bambara groundnuts and these strains could be considered as potential bioinoculant strains for sustainable production of Bambara groundnut in Nigeria. However, there is a need to conduct field experiments to ascertain the applicability of these strains.

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