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Full Length Article

Effect of *Bradyrhizobium japonicum* Strains on the Performance of Accessions of Bambara Groundnut (*Vigna subterranea*)

Tope Daniel Bitire[1,2], Michael Abberton[2], Olaniyi Ajewole Oyatomi[2] and Olubukola Oluranti Babalola[1*]
[1]*Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Private bag X2046 Mmabatho, 2735, South Africa*
[2]*Genetic Resources Center, International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo Road, Ibadan, Nigeria*
[*]For correspondence: olubukola.babalola@nwu.ac.za
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Abstract

Pot experiments were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, in 2018 and 2019 in the glasshouse and field experiments in two different geographical locations: Ibadan and Ikenne, August-December 2019 and 2020 cropping season in Nigeria. The use of inorganic Nitrogen fertilizer (N fertilizers) after use often leads to environmental contamination and adverse effect on soil health. Also, the inability of farmers in the tropics to procure inorganic fertilizer to amend legume as a starter dose resulted to the use of bacteria strains that are easily affordable and improved the growth and developments of legumes. In this study, ten accessions of Bambara groundnut: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475 and TVSu-305 were inoculated with broth culture of four *Bradyrhizobium japonicum* strains (*B. japonicum* strains): FA3, USDA110, IRJ2180A and RACA6. Nitrogen fertilizer (Urea, 20 kg ha^{-1}) was applied to uninoculated seedlings 2 weeks after planting (WAP) and an uninoculated control (zero inoculation and zero fertilizer application) in the glasshouse, while six seeds of each accession were coated with each of the *B. japonicum* strains before planting on the field. Data were collected on the growth traits, biomass yield and nutrient uptake in the glasshouse and on the field and were subjected to analysis of variance, and means were separated using the Duncan multiple range test (DMRT) at ($P < 0.05$) level of probability. *B. japonicum* strains, FA3 was found to improve the growth traits and nutrients uptake significantly by 40% increase especially when applied on the field as inoculant and was able to out-compete other strains and N fertilizer applied in the study particularly for TVSu-1739, TVSu-378 and TVSu-787 accessions under both glasshouse and the field conditions while the inoculation significantly enhanced the yield of TVSu-1698 (1202.5 kg ha^{-1}) than other inoculated accessions on the field. The study revealed the importance of the inoculations of bacteria strains over the inorganic N fertilizer as a starter dose to improve the performance of legumes.

Keywords: Underutilized legume; Beneficial soil microorganism; Inoculation; Plant growth nutrition; Fertilization

Introduction

Bradyrhizobium are beneficial symbionts that are highly present in terms of its distribution as a free-living bacteria in different habitats and in association with leguminous plants (Sprent *et al*. 2017) unlike other N_2-fixing microsymbionts, it exhibits a life cycle (bipartite) which varies between the free-living state in soils and as a symbiotic partner inside root nodules of legumes (Jaiswal and Dakora 2019). The inoculation of the *Bradyrhizobium* strains often leads to the supply of the nitrogen needed by plant through (Hungria and Mendes 2015) biological nitrogen fixation (BNF) without the addition of chemical N fertilizer which eventually leads to economic savings (Hungria and Mendes 2015), decrease in emission of greenhouse gases, and reduce risk of contamination of surface and ground water with nitrate (Hungria and Mendes 2015; Moraes *et al*. 2017).

Bradyrhizobium are major components of soil microbiota which enhanced the improvement of soil fertility, plant growth nutrition and contributes an important role in organic agriculture which eventually led to the reduced application of inorganic fertilizers and agrochemicals. it usually colonize roots of leguminous plants and form symbiotic association that leads to utilization of water and adequate nutrient uptake (Gitonga *et al*. 2021). The use of beneficial soil microorganisms in organic farming, is an improving and promising smart-technology that could be used to reduce the intensive use of inorganic fertilizers to amend leguminous crops (Fasusi *et al*. 2021). The roles of the these bacteria have been marginalized in modern agriculture since microbial communities in conventional farming systems have been modified due to tillage (Kerry *et al*. 2018; Yadav *et al*. 2018) and high inputs of inorganic fertilizers, herbicides and

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pesticides (Malhotra *et al*. 2015; Prashar and Shah 2016).

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an indigenous and underutilized African leguminous crop, grown for human and animal consumption. It is ranked third in importance after other legumes like groundnut (*Arachis hypogea*) and cowpea (*V. unguiculata*) in Africa (Mubaiwa *et al*. 2017). Bambara groundnut seed contains carbohydrate (58.3%), protein (23.7%) and fat (4.3%) with high amount of methionine content than other leguminous crop (Oyeyinka *et al*. 2018). It has the ability to produce high yields even in low nutrient soils and where there is drought stress due to low soil moisture (Ibny *et al*. 2019). Bambara groundnut can fix about 4 to 200 kg N ha^{-1} (Mohale *et al*. 2014) with symbiotic relationship with soil bacteria called 'rhizobia (Puozaa *et al*. 2017). As rhizobial species nodulating, most legumes can vary between geographical locations, it is important to continuously explore new geographic regions to identify rhizobia that are capable of effectively nodulating and promoting the growth of Bambara groundnut (Ibny *et al*. 2019). Some of the most promising strains for inoculation of Bambara groundnuts established were; BAMKis12, BAMKis8, BAMKis4, BAMKar3 (*Bradryrhizobium* spp.), BAMKbay8 and BAMsp3 (*Burkholderia* spp.) which produced highly effective nodules and high plant biomass value, (Benson and Fredrick 2019).

The deficiency of N in soil often result to leaf senescence, lower yield production, and biomass in plants (Kant *et al*. 2011). High cost of procuring the inorganic N fertilizer by the tropical farmers is a major challenge and most farmers that can afford usually apply below manufacturer recommendation. Very little information is available on the biodiversity of rhizobia nodulating Bambara groundnut in African soils, except for a few studies which have shown that Bambara groundnut is nodulated by species of the genus *Bradyrhizobium*. Therefore, this study was conducted to evaluate the effect of inoculation of *B. japonicum* strains with reduced cost to amend and to improve the growth traits, nutrient uptake, and yield of different accessions of Bambara groundnut that differs in genetic composition.

Materials and Methods

Seed scarification

The seeds planted in the glasshouse were scarify using scalpel, the seeds of the accessions were introduced into a beaker containing 3% w/v (Sodium hypochloride), the solution was decanted and rinse with sterile water and 90% of ethanol was added for 30 sec and was decanted and also rinse after 1 min with sterile water (Davis *et al*. 1991).

Authentication of the strains

Seeds were sterilized, using 3% w/v sodium hypochloride, planted in sterile carrier materials and allowed to grow for 10 days before inoculation with broth containing about 1×10^9 cfu/mL of rhizobia cell. Plants were allowed to grow for about six to eight weeks to check for infectivity of rhizobia isolates. Most effective isolates were selected and used for pot experiments and field experiment (Vincent 1970).

Broth preparation

To prepare 1 liter of solution, mannitol 10 g, yeast extract powder 0.5 g, potassium phosphate 0.5 g, magnesium sulphate 0.2 g, sodium chloride 0.1 g, agar powder 15 g. The mixture was dissolved in a conical flask with 1000 mL of distilled water and stir to homogenize the solution. The pH was adjust to 6.8 and sterilize using the autoclave at 121°C for 15 min at a pressure of 15 psi, place in the water bath to adjust temperature to 47°C and was poured in sterile petri dishes (Vincent 1970).

Determination of most probable number

Rhizobia were isolated from nodules on Congo red agar (Woomer 1994) using spread plate method. Two undamaged nodules samples were picked from each plant of Bambara groundnut and placed in sterile water for about 15 to 20 min to rehydrate them after which they were surface sterilized using 3% sodium hypochlorite for 3 min. They were then rinsed with sterile water after which they were further sterilized with 95% ethanol and then rinsed with six changes of sterile water (Woomer 1994). The nodules were then transferred into sterilize petri-dishes, crushed with flamed glass rod and mixed with a few drops of sterile water. A loop full of the crushed nodule were streaked on Congo red agar and then incubated at 28°C for 5–7 days isolates were purified and identified.

Nutrient solution preparation

To prepare 1 L of macronutrient, 100 g of calcium phosphate, 20 g of potassium phosphate, 20 g of hydrated magnesium sulphate, 20 g of sodium chloride, 10 g of iron (11) chloride are dissolve in 100 mL of distilled water and stir till the solution is homogenous. To prepare 1 L of micronutrient solution, 2.86 g of orthoboric acids, 1.81 g of magnesium chloride, 0.22 g of zinc sulphate, 0.025 g of sodium molybdate were dissolve in 1000 mL of distilled water and stir (Steiner and Soil 1961).

Pre-sowing soil analysis used in pot and field experiments

The pH of the soils used in the glasshouse in both seasons was neutral, the pH of the soil in Ikenne was acidic in nature in both seasons while in Ibadan, pH was slightly acidic. The % organic carbon ranged from 0.25 to 0.40 in both glasshouse and on the field which shows the soil used is

normal. The phosphorus in the soil used in the glasshouse is low, higher % of P was recorded in the soil used in the field in both locations and seasons. The % of K present in the soil used in both glasshouse and in the field in both seasons shows availability in moderate quantities. The calcium in the soil used in the glasshouse were extremely high, while the calcium in the field in Ibadan were considered to be normal (Table 1). The Mg present in the soils used for the glasshouse experiments were high in both sterile and non-sterile soil in both seasons. Higher Mg values were obtained in Ikenne in both season while lower quantities of Mg were obtained in Ibadan in both seasons. The particle size analysis was determined by the (hydrometer method). The soil textural class shows that both the soil used in the glasshouse and on the field is a loamy sand (Table 1).

Pot experiment

An experiment was carried out in the glasshouse in 2018 and 2019 at the International Institute of Tropical Agriculture (IITA) headquarter, Ibadan, Nigeria [Latitude (Lat) 7° 22´ 30 N and Longitude (long) 3° 45´ 54 E] to determine the growth and nutrient uptake of different inoculated accessions. The soil used in the glasshouse was carefully sieve through 3 mm sieve and was sterilized at 121°C for 1 h using the autoclave.

Ten accessions of Bambara groundnut namely TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475 and TVSu-305 were selected from the International Institute of tropical Agriculture (IITA) Gene bank and were inoculated with each *B. japonicum* strains: FA3, RACA6, USDA110 and IRJ2180A containing 2.8×10^7, 7.2×10^6, 4.3×10^7, 1.4×10^7 cfu/mL respectively, N fertilizer (Urea, 20 kg/ha) and an uninoculated control. The choice of the *B. japonicum* strains depend on the availability and authencity (ability to nodulate bambara groundnut) and were inoculated to the seedlings of the ten accessions of Bambara groundnut in the glasshouse (Argaw 2014).

The experiment was laid out in a completely randomized design (CRD) with factorial arrangement using sterile and nonsterile soil and was replicated three time. The seeds were surface sterilized and two seeds were sown into 10 kg pot containing the sterile and nonsterile soil in the glasshouse. N free nutrient solution and sterile water was added to each pot at regular interval (twice a week) (Afzal *et al*. 2010). Data regarding growth traits and nutrient uptake were collected at 10 WAP while biomass yield was recorded at 50% flowering after drying at 72°C for 48 h (Argaw 2014).

Data collection

All data collected on growth parameters and biomass yield were taken base on the descriptor of Bambara groundnut, International Plant Genetic Resources Institute, (IPGRI) Rome (Italy); International Institute of Tropical Agriculture; International Bambara Groundnut Network (BAMNET). Vegetative traits recorded in this study from the descriptor include (Peduncle length (cm), number of leaves, terminal leaflet length (cm), terminal leaflet width (cm), petiole length, plant spread (cm), plant height (cm), numbers of stem and branches per plant.

Determination of nitrogen in plant: preparation of digest

A 0.2 g of plant sample was weighed into a digestion tube, then 2.5 mL of acid mixture and 3 mL of hydrogen peroxide was added to the plant samples and was stirred and place on the digesting block at 150°C for 30 min in the fume cabinet and temperature was increased to 330°C. The sample was digested until extract turn colourless, sample was removed from the fume cabinet and place in the rack to cool and top up with 50 mL of distilled water (Anderson and Ingram 1989). All digest were diluted 1:9 (w/v) with distilled water and 0.2 mL of sample digested was taken with a micropipette and was place in a clearly labelled test tube and 5.0 mL of the reagent N_1 and N_2 was added, vortex and allow to stay for 2 h and absorbency was measured at 650 nm. The blue is stable for 10 h and the concentration of N in the solution was measured. Nitrogen concentration in the sample materials expressed in %N is calculated as:

$$\%\ N = \frac{(A-B) \times V \times K}{H^2 \times W \times al}$$

Where A = Concentration of N in the solution (n/mol), B = concentration of N in the blank (n/mol), V = total volume at the end of analysis procedure (mL), W = weight of dried samples (g), H= 1000, K = 100 and al = aliquot of the solution taken (mL).

N_1= 34g sodium salicylate, 25 g sodium citrate and 25 g sodium tartrate in 75 mL water

N_2= 30 g sodium hydroxide,10 mL sodium hypochlorite and make up to 1 L (Lindner and Harley 1942).

Field experiments

Field experiments was carried out at two different geographical locations: Ibadan (7° 38'N, 3° 89'E) and Ikenne (6° 86'N, 3° 71'E), Nigeria in 2019 and 2020 cropping seasons to determine the growth, nutrient uptake, and yield of inoculated accessions of Bambara groundnut. Each field were prepared using a tractor driven plough and harrowed to remove plant debris and 2 m plot were made with a spacing of 25 cm between plot and 1m between each rep. The experiments were arranged in randomize complete block design (RCBD) on the field in both locations and cropping season and was replicated three (3) times.

Ten accessions of Bambara groundnut: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739 TVSu-710, TVSu-365, TVSu-475 and TVSu-305 used in the glasshouse were also used for field studies and

were randomly selected from the International Institute of Tropical Agriculture (IITA) Gene bank. Six seeds from each accession were coated with each of the *B. japonicum* strains FA3, RACA6, USDA110, and IRJ2180A containing 2.8×10^7, 7.2×10^6, 4.3×10^7, 1.4×10^7 Cfu/mL respectively, N fertilizer (Urea, 46%) and an uninoculated control. Seed of accessions were coated with the *B. japonicum* strains using gum arabic and allowed to dry with the seeds (Nodumax IITA) before planting on the field at both locations and cropping seasons. Regular weeding was done manually using hoe to remove weeds.

Data were collected at 12 weeks after planting (WAP) on growth traits and at 50% flowering on nutrient uptake. Data recorded on growth traits include peduncle length (cm), terminal leaflet length (cm), terminal leaflet width (cm), petiole length, plant spread (cm) and plant height (cm). Data was taken using a well calibrated meter rule, while the data on the numbers of stem, number of leaves, number of branches and on flowering recorded was obtained by counting. Data on chlorophyll content of accessions of Bambara groundnut was obtained using the spad meter. Also, data on yield was obtained using the weighing balance.

Statistical analysis

Data collected were subjected to four-way ANOVA (Analysis of Variance): pot (Accessions, strains, soil status and season), field (Accessions, strains, locations and seasons) statistical analysis system (SAS) package 9.4 and means were separated by using Ducan Multiple Range Test (Dmrt) at $P < 0.05$ (Zatybekov *et al*. 2017).

Results

Microbial population of the soil used for both pot and field experiment

The non-sterilize soils used in the glasshouse experiments, based on microbial analysis, indicated the presence of low rhizobia population during 2018 season (1.28×10^2) and high in 2019 (1.53×10^9) season (Table 2). However, in Ikenne field, rhizobia population was high *i.e*., 2.03×10^9 cfu/mL and 2.47×10^7 cfu/mL in 2019 and 2020 season, respectively. In Ibadan field, the rhizobial population was also high *i.e*., 1.83×10^5 cfu/mL and 3.05×10^7 cfu/mL in 2019 and 2020 season, respectively (Table 2).

Growth traits and nutrient uptake of inoculated accession under glasshouse conditions

At 10 weeks after planting (WAP), analysis of variance (ANOVA) indicated that significant differences were recorded among accessions, soil status (sterile and non-sterile soil) and seasons in the growth traits recorded (Table 3). These significant differences among the accessions, soil status and seasons was due to the inoculation of *B. japonicum* strains. While non-significant difference was recorded among the strains inoculated in all the growth traits under glasshouse conditions. The non-significant differences recorded among strains inoculated under glasshouse conditions showed that the *B. japonicum* strains were nearly equal in performance. Furthermore, non-significant difference was recorded in growth traits regarding the interaction of accessions with soil status, strains with soil status, and accessions with strains with exception to chlorophyll content of accessions (Table 3).

ANOVA indicated that interactive effect of accessions and seasons had significant effect on the number of stem (NOS) and number of leaf (NOL) (779.31** and 6041.09*). Likewise, interaction of strains and seasons (620.32* and 5978.13*), accessions × strains × soil status × season (368.90** and 8737.41**) had significant effect on NOS and NOL of Bambara groundnut (Table 3). However, all two-way and three-way interactions were non-significant on entire growth-related traits (plant height, canopy spread, and chlorophyll) of Bambara groundnut (Table 3).

ANOVA indicated that *B. japonicum* strains had significant effect on Bambara groundnut accessions on days to 50% flowering (399.45**) and nutrient uptake (%N and %P) at flowering and at harvest (Table 4). Moreover, non-significant difference was recorded among the strains inoculated in the number of days to 50% flowering (261.39^{ns}), % P uptake at flowering (0.005^{ns}) and at harvest (0.004^{ns}) under glasshouse conditions (Table 4). However, significant difference was recorded among the strains inoculated regarding N uptake at flowering (20.87^{**}) and at harvest (16.07**) under glasshouse conditions due to the inoculation of *B. japonicum* strains (Table 4). Moreover, significant difference was recorded in Pflw and PHvst in the soil status (0.17** and 0.19**) and season (1.06** and 0.23**) due to the inoculation of *B. japonicum* strains. Nevertheless, the interactions of accessions with soil status, strains with soil status, and accessions with strains, soil status and season had non-significant effect on nutrients uptake recorded at 50% flowering under glasshouse conditions (Table 4). However, significant difference was also recorded regarding interaction of accessions with strains on number of days to 50% flowering (282.45**), accessions with season on the P uptake at flowering (0.01**) and strains with season on N uptake at flowering (0.78**) and at harvest (Table 4).

Growth traits, nutrient uptake and yield of inoculated accessions on the field

Significant difference was recorded in Ibadan and Ikenne in both seasons in TVSu-1739 in the plant height (25.53^a), terminal leaf length (13.89^a), terminal leaf width (2.75^a), and petiole length (2.29^a) compared to other inoculated accessions (Table 5). Likewise, TVSu-378 strain recorded higher number of branches, number of stems and number of

Table 1: Physiochemical properties of soil used for the study in the glasshouse and on the field

Experiment	Pot Experiment				Field Experiment			
Year	2018	2018	2019	2019	2019	2020	2019	2020
Soil status/ Location	Sterile Soil	Non sterile Soil	Sterile soil	Nonsterile soil	Ikenne	Ikenne	Ibadan	Ibadan
pH (H_2O)	7.43	7.39	7.1	6.81	4.46 ± 0.08	4.91 ± 0.09	6.84 ± 0.1	7.13 ± 0.11
N (%)	0.05	0.04	0.05	0.10	0.073 ± 0.02	0.121 ± 0.01	0.150 ± 0.013	0.119 ± 0.02
OC (%)	0.38	0.40	0.03	0.25	0.329 ± 0.07	0.297 ± 0.01	0.407 ± 0.10	0.336 ± 0.06
Bray P (mg kg^{-1})	3.30	3.71	2.55	3.71	13.23 ± 4.26	22.46 ± 3.43	15.352 ± 3.74	52.84 ± 6.67
Sand (%)	81.00	83.00	77.0	81.00	76.00 ± 1.16	76.00 ± 1.16	83.00 ± 1.16	82.00 ± 0.0
Clay (%)	13.00	11.00	19.00	11.00	16.00 ± 1.16	20.00 ± 2.00	10.00 ± 0.00	8.00 ± 0.00
Silt (%)	6.00	6.00	4.00	8.00	6.00 ± 1.15	3.00 ± 1.15	7.00 ± 1.15	10.00 ± 0.0
Ca (Cmol/kg)	6.74	5.92	8.53	8.63	3.530 ± 2.50	1.505 ± 0.24	1.216 ± 0.10	1.101 ± 0.30
Mg (Cmol/kg)	6.74	5.92	0.20	0.20	0.80 ± 0.37	0.404 ± 0.02	0.075 ± 0.01	0.158 ± 0.05
K (Cmol/kg)	0.24	0.22	0.27	0.29	0.560 ± 0.24	0.242 ± 0.06	0.137 ± 0.02	0.201 ± 0.02
Na (Cmol/kg)	0.06	0.06	0.08	0.08	0.076 ± 0.01	0.082 ± 0.00	0.055 ±0.02	0.057 ± 0.01
ECEC (Cmol/kg)	13.79	12.12	9.08	9.21	4.96 ± 1.56	2.23 ± 0.57	1.29 ± 0.56	1.47 ± 0.48
Zn (mg kg^{-1})	3.24	2.66	9.91	9.39	1.964 ± 1.67	1.196 ± 0.19	1.19 ± 0.10	0.66 ± 0.04
Cu (mg kg^{-1})	0.95	0.49	0.64	0.70	1.167 ± 0.62	2.045 ± 0.19	0.86 ± 0.11	0.97 ± 0.22
Mn (mg kg^{-1})	266.0	286.2	214.7	222.1	12.15 ± 10.5	116.7 ± 3.31	244.20 ± 18.4	383.6 ± 33.4
Fe (mg kg^{-1})	17.19	17.19	85.54	94.01	25.58 ± 7.47	88.29 ± 4.08	133.33 ± 3.33	114.4 ± 3.85
Glomus	-	159	-	145	106	117	135	152
acaulospora	-	211	-	200	192	185	202	208
enthrophospora	-	30	-	36	25	28	34	32
Spore/100gdwt	-	401	-	406	380	392	412	432
Soil textural class	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy Sand	Loamy sand

Table 2: Microbial population of the soil used for both pot and field experiment

Experiment site	year	Soil status/Location	colony forming unit
Glasshouse	2018	Non sterile soil	1.28×10^2
	2019	Non sterile soil	1.53×10^9
Field	2019	Ibadan	1.83×10^5
	2019	Ikenne	2.03×10^9
	2020	Ibadan	3.05×10^7
	2020	Ikenne	2.47×10^7

Table 3: Analysis of variance (ANOVA) of accessions of Bambara groundnut inoculated with *B. japonicum* strains on the growth traits at 10WAP under glasshouse conditions in both seasons

Source of variation	DF	PLH (cm)	CS (cm)	NOS/plant	NOL/plant	CHPY
Accessions	9	407.97**	92.31**	1845.74**	17593.64**	417.89**
Strains	5	12.017^{ns}	1.69^{ns}	274.76^{ns}	526.42^{ns}	95.11^{ns}
Soil Status	1	841.42**	159.41**	15797.87**	160776.32**	384.79^{ns}
Season	1	4969.64**	2134.50**	5037.88**	36155.86**	6896.92**
Rep	2	28.48^{ns}	17.25^{ns}	1673.01**	8792.17*	82.23^{ns}
Accessions*Strains	45	30.38^{ns}	8.90^{ns}	319.78^{ns}	2905.64^{ns}	232.18**
Accessions*Soil status	9	26.95^{ns}	10.50^{ns}	293.01^{ns}	3224.99^{ns}	175.16^{ns}
Accessions*season	9	33.19^{ns}	5.79^{ns}	779.31**	6041.09*	148.13^{ns}
Strains*Soil Status	5	6.08^{ns}	4.47^{ns}	280.94^{ns}	3314.61^{ns}	240.49^{ns}
Strains*seasons	5	52.34^{ns}	14.37^{ns}	620.32*	5978.13*	254.50^{ns}
Acc*Strain*soil*seas	131	32.04^{ns}	7.51^{ns}	368.90**	8737.41**	150.86^{ns}

***=** Significant; ** = Highly significant; ns = Non-significant, DF = Degree of freedom; NOB = number of branches, NOL= Number of leaves, CS = Canopy spread; PLH; Plant height; NOS = Number of stem; CHPY= Chlorophyll; WAP = Weeks after planting

leaves per plant at both locations and seasons compared to other inoculated accessions. Moreover, strains TVSu-506 and TVSu-305 observed higher leaf area and chlorophyll contents, respectively (Table 5).

Analyzed data indicated that different *B. japoniocum* strains inoculation had significant effect on entire growth-related traits of Bambara groundnut on the field in both locations and seasons. Among different strains tested, FA3 observed higher plant height, number of branches and stems/ plant, leaf area, terminal leaf length and width, number of leaves/plant and chlorophyll contents compared with all other strains and control (Table 6).

Inoculation of different *B. japoniocum* strains significantly improved the nutrient uptake and yield of inoculated accessions of Bambara groundnut. Higher N contents were recorded at flowering and harvest in TVSu-1739, TVSu-378 and TVSu-787 accessions respectively in Ibadan and Ikenne in both seasons and were significantly higher than other inoculated accessions of Bambara groundnut (Table 7). Likewise, high P uptake was recorded at flowering and at harvest in TVSu-787 and TVSu-1606, TVSu-378 and TVSu-787, respectively (Table 7). Among

Table 4: Analysis of variance reflecting accession of Bambara groundnut inoculated with *B. japonicum* strains on the number of days to 50% flowering, %N and %P at flowering and harvest under glasshouse conditions in both season

Sources of variations	DF	Days to 50% Flw	P@Flw (%)	N@Flw (%)	N@Hvst (%)	P@Hvst (%)
Accessions	9	399.45**	0.012**	0.98**	0.67**	0.02**
Strains	5	261.39ns	0.005ns	20.87**	16.07**	0.004ns
Soil Status	1	53.06ns	0.17**	0.28ns	3.01**	0.19**
Seasons	1	792.23**	1.06**	1.28*	0.019ns	0.23**
Rep	2	196.78ns	0.012**	0.17ns	0.56ns	0.02**
Accessions*Strains	45	282.45**	0.003ns	0.38ns	0.24ns	0.002ns
Accessions*Soil Status	9	226.80ns	0.003ns	0.17ns	0.16ns	0.004ns
Accessions*season	9	226.80ns	0.01**	0.78**	0.25ns	0.003ns
Strains*soil status	5	99.12ns	004ns	0.43ns	0.21ns	0.01ns
Strains*season	4	189.71ns	0.003ns	0.48ns	0.85**	0.003ns
Acc*Strain*soil*season	131	163.25ns	0.002ns	0.29ns	0.003ns	0.003ns

* = Significant; ** = Highly significant; ns = Non-significant; DF = Degree of freedom; Flw = Flowering; Hvst = Harvesting; %P = Phosphorus; %N = Nitrogen; @ = at

Table 5: Effect of the inoculation of *B. japonicum* strains on the growth traits of accessions of Bambara groundnut at 12WAP in (Ibadan and Ikenne) in both seasons

Accessions	PLH (cm)	NS/plant	NB/plant	LA (cm^2)	NOL/plant	TLL (cm)	TLW (cm)	PEL (cm)	CPHY
TVSu-506	24.01ab	55.08d	50.90e	21.05a	160.47f	5.75b	2.44b	1.66c	31.85d
TVSu-1739	25.53a	50.04e	45.28f	18.82b	147.75g	13.89a	2.75a	2.29a	36.57b
TVSu-305	20.42c	69.64c	62.76c	14.89c	209.36c	5.75b	2.64ab	1.45d	41.99a
TVSu-787	21.76bc	79.43b	72.39b	12.15d	234.18b	5.51bc	2.00d	1.78b	33.13c
TVSu-378	18.84d	95.00a	79.75a	12.68d	278.75a	5.10cd	2.20cd	1.43d	31.83d
TVSu-1698	22.70b	70.79c	65.09c	15.98c	202.89d	5.89b	2.69a	1.47d	35.04b
TVSu-710	18.77d	39.40g	34.94h	12.76d	121.68g	5.35bcd	2.22cd	1.41d	31.05d
TVSu-475	21.29bc	62.90d	56.61d	13.92d	183.75e	5.83b	2.34c	1.38e	31.70d
TVSu-365	18.85d	78.62b	65.27c	10.20e	210.12c	4.45d	1.74e	1.39e	27.84f
TVSu-1606	17.23e	47.01f	39.71g	12.61d	133.51h	4.89d	2.08d	1.22f	29.38e

Mean with the same letter are not significantly different at $P < 0.05$ level of probability according to DMRT
PLH = Plant height; NS = Number of stem; NB = Number of branches; LA = Leaf area; NOL = Number of leaves; TLL = Terminal leaf length; TLW = Terminal leaf width; PEL = Petiole length; CHPY = Chlorophyll

Table 6: Effect of the inoculation of *B. japonicum* strains on the growth traits of accessions of Bambara groundnut at 12WAP in (Ibadan and Ikenne) in both season

Strains	PLH (cm)	NB/plant	NS/plant	LA (cm^2)	NOL/plant	TLL (cm)	TLW (cm)	PEL (cm)	CS (cm)	CPHY units	PL (cm)
FA3	22.71a	62.73a	70.11a	15.74ab	209.25a	6.00b	2.56a	1.73a	12.21a	34.53a	12.69a
USDA110	20.23b	54.68bc	60.83b	12.93b	177.48b	5.44c	2.22c	1.49c	10.37bc	33.26ab	9.89b
N	18.99c	48.20d	53.59c	13.02b	156.83c	5.18c	2.21c	1.47c	9.97c	31.48b	9.03a
RACA6	20.78b	55.64b	61.15b	17.09a	178.01b	5.41c	2.36b	1.56bc	10.99b	33.35ab	9.88b
IRJ2180A	20.38b	51.08cd	58.84b	13.45b	167.07bc	9.75a	2.22c	1.45c	10.46bc	30.96b	9.46a
Control	21.07b	52.18bcd	58.03bc	14.83ab	167.44bc	5.90b	2.46ab	1.64ab	11.06b	34.09a	9.48b

Means with the same letter are not significantly different at $P < 0.05$ level of probability according to DMRT
PLH = Plant height; NS = Number of stem; NB = Number of branches; LA = Leaf area; NOL = Number of leaves; TLL = Terminal leaf length; TLW = Terminal leaf width; PEL = Petiole length; CHPY = Chlorophyll; PL = Peduncle length

Table 7: Mean separation reflecting nutrient uptake of accessions of Bambara groundnut inoculated with *B. japonicum* strains at flowering, harvest and yield (Ibadan and Ikenne) in both season

Accessions	N@Flw (%)	P@Flw (%)	N@Hvst (%)	P@Hvst (%)	Yield/plot (g)	Yield (kg ha^{-1})
TVSu-1739	2.09a	0.19b	1.67bc	0.12b	58.33ab	784.3bcd
TVSu-378	2.05ab	0.21ab	1.80a	0.15a	27.43de	403.1def
TVSu-305	2.03abc	0.17d	1.62cd	0.12bc	64.15ab	825.2abc
TVSu-710	2.02a-d	0.17d	1.68b	0.12bcd	30.06cde	364.7ef
TVSu-365	2.01bcd	0.17d	1.48f	0.11d	60.89ab	812.7abc
TVSu-1698	2.01bcd	0.19bc	1.53ef	0.11d	80.92a	1205.5a
TVSu-787	2.00bcd	0.21a	1.75a	0.14a	39.99b-e	669.6b-e
TVSu-475	1.99bcd	0.17cd	1.52ef	0.11d	56.31b	871.1ab
TVSu-506	1.96cd	0.19b	1.56ed	0.12bcd	42.42bcd	523.3c-f
TVSu-1606	1.95d	0.21a	1.53ef	0.11cd	51.08bc	733.5b-e

Means with the same letter are not significantly different at 5% level of probability using DMRT
N = Nitrogen; P = Phosphorus; Flw = Flowering; Hvst = Harvest; @ = at

the inoculated accessions, higher yield was recorded by TVSu-1698 (1205.5 kg ha^{-1}) compared to other inoculated accessions at both locations and seasons due to the inoculation of *B. japonicum* strains that enhanced the yield components (Table 7).

Discussion

In general, the results obtained in this study, eventually revealed the relevance of *B. japonicum* strains to improve the growth traits and nutrient uptake of accessions of

Bambara groundnut under glasshouse (sterile and non-sterile soil) conditions. Also, inoculation of *B. japonicum* strains enhanced growth traits, nutrient uptake and yield of accessions of Bambara groundnut in the field in both locations and seasons over the N fertilizer application (Table 6 and 7).

Significant differences were recorded among the inoculated accessions in the growth traits and nutrient uptake in the glasshouse and on the field on yield components in both locations and seasons. Among the *B. japonicum* strains inoculated to accessions, FA3 showed higher significant difference compare to other strains, N fertilizer application, and uninoculated control which shows that significant differences exist among the *B. japonicum* strains (Table 6). The result obtained in this study is related to the result recorded by (Tarekegn *et al.* 2017) when cowpea upon inoculation with *Bradyrhizobium* on the field, significantly enhanced the growth traits, nodulation, and yield of cowpea when compared with the uninoculated control.

Furthermore, the nutritional benefit recorded in both studies was as a result of easy translocation of nutrients from the soil by the strains to a point where the root can intercept the nutrients for optimum growth and developments. The result obtained on nutrition is similar to the findings of (Biswas *et al*. 2000) when inoculation with rhizobia resulted to larger amount of N uptake when compared with the uninoculated control. Superior performance was recorded among inoculated accessions of Bambara groundnut, as the response to the *B japonicum* strains varies probably, due to genetic compositions of accessions that differs. The variability recorded among inoculated accessions of Bambara groundnut correlates with the findings of (Nyoki and Ndakidemi 2014) where varying responses were observed among varieties of soybean that were inoculated on the field.

It seemed apparent from the responses obtained in this study that inoculation of FA3 strains mostly supported the growth, nutrition and yield of accessions of Bambara groundnut. FA3 strains perform better compare to other *B. japonicum* strains inoculated, N fertilizer applied, and uninoculated control in Ibadan and Ikenne in both seasons (Table 6). The results obtained correlates with the result of the research study recorded by Kaschuk *et al*. (2016), in which neither basal nor topdressing of N application improved yields of determinate and in determinate soybean cultivars, but the sole inoculation of *Bradyrhizobium* was enough to supply all N required by soybean plants. The result of this study also agrees with the research conducted by (Hungria and Mendes 2015), that embraced rhizobia inoculation and zero N fertilizer application to enhance legume production.

Our findings reveals that the inoculation of the *B. japonicum* strains can help to improve the growth, nutrient uptake and yield of Bambara groundnut with reduced cost as against the use of inorganic N fertilizers which is very expensive to procure and also contaminate the soil after use (Cordeiro and Echer 2019). Also, in another study conducted inoculating five cowpea varieties with five Bradyrhizobium isolates, result revealed that *Bradyrhizobium* inoculation improve the growth, biomass, and nodulation performance of the varieties of cowpea, Ayalew and Yoseph (2020). Bambara groundnut in this study, refuses to pod in the glasshouse in both seasons, which also therefore, limits it production in most research to growth traits and nutrition under glasshouse conditions.

Conclusion

Results of this study unveiled that the inoculation of *B. japonicum* strains can help to improve the growth traits, nutrient uptake and yield of Bambara groundnut accessions under glasshouse and field conditions. Resultantly it can reduce reliance on inorganic N fertilizer which most tropical farmers cannot afford and for those that can afford apply at low rate than recommended. Much effort is needed to introduce the inoculant to the farming community (farmers). It is therefore important to introduce and recommend the cheap and friendly technology to the poor farmer's communities of the nation. FA3 therefore, can be introduce to the farmers to enhance optimum production of Bambara groundnut.

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Author Contributions

TDB and MA planned the experiments, TDB and OOB interpreted the results, MA, OOB and OO supervision and TDB statistically analyzed the data and write up.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Ethics Approval number: N W U - 0 1 2 1 7 - 1 9 - A 9

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