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Effect of *Bradyrhizobium japonicum* Strains and Inorganic Nitrogen Fertilizer on the Growth and Yield of Bambara Groundnut (*Vigna subterranea (L.) Verdc*) Accessions

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This study was set up to compare the inoculation of *Bradyrhizobium japonicum* strains and the application of nitrogen (N) fertilizers (urea with 46% nitrogen) on the growth and yield of Bambara groundnut accessions. The study results suggest that the benefits of *Bradyrhizobium japonicum* (*B. japonicum*) strain inoculation are greater and that the strain could reduce reliance and the excess amount spent by farmers to procure inorganic fertilizers and avoid the negative effect of *N* fertilizer on the environment after its use. Field studies were conducted in two different geographical locations, in Ibadan (Ib) and Ikenne (Ik), Nigeria, during the rainy season between August and December in 2019 and 2020. The experiment was arranged in a randomized complete block design (RCBD) in both locations and seasons and was replicated three times, with each block representing each replicate. It had a 10 × 6 factorial arrangement with one block holding the 10 accessions of Bambara groundnut inoculated with four *B. japonicum* strains. The second block had *N* fertilizer application and the third control block was without inoculation or fertilizer application. The 10 accessions of Bambara groundnut used in the study were as follows: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475, and TVSu-305. Six seeds of each accession were coated with each of the four *B. japonicum* strains, namely, FA3, USDA110, IRJ2180A, and RACA6, before planting them in the field in both locations during the rainy season. In the next block, urea as *N* fertilizer (46% nitrogen) was applied to the uninoculated seedlings of accessions of Bambara groundnut 2 weeks after planting (WAP). The third block was the control with zero inoculation and zero fertilizer application. Data collected were subjected to an analysis of variance and mean and were separated using Duncan's Multiple Range Test (DMRT) at a $p > 0.05$ level of probability. It was found that FA3 inoculation significantly enhanced the growth traits of the accessions than other strains and *N* fertilizer application. In both locations and seasons, at 7 weeks after planting (WAP) and 12 WAP, plant height (19.54 and 22.71 cm), number of branches (33.63 and 62.77), number of leaves (116.54 and 209.25), terminal leaf length (5.62 and 6.00 cm), and width (2.09 and 2.56 cm) were recorded. The yield and yield components recorded at harvest

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were as follows: pod length (13.27 cm), pod width (9.08 mm), seed length (9.39 mm), seed width (6.92 mm), weight of 100 seeds (56.85 g), and yield/ha (750.72 kg). The yield and yield components were also significantly influenced by the inoculation of FA3 and RACA6 than other inoculated strains and *N* fertilizer application in both locations and seasons.

Keywords: underutilized legume, inoculation, plant trait, bacterial strains, inorganic fertilizer

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a leguminous crop usually cultivated in sub-Saharan Africa and some parts of Asia but is now grown all over the world. It can thrive in all kinds of soil, especially in marginal soil (Mandizvo and Odindo, 2019; Mayes et al., 2019; Soropa et al., 2019). *Bradyrhizobium* usually establishes mutual symbiosis with legumes by creating root nodules and fixing atmospheric nitrogen for the host plant and can be used as an inoculant for improving crop productivity (Jaiswal and Dakora, 2019). Also, inexpensive microbial inoculants help in improving plant growth, reducing heavy metal contaminations, and controlling phytopathogens to enhance sustainable agriculture (Fasusi and Babalola, 2021). However, bacterial strains used in inoculation may not dominate during nodule formation due to competition with indigenous strains in the crop field (Suzuki et al., 2014). On the other hand, applying *N* fertilizers helps improve the nutrition of the plant (Islam et al., 2018). It is also very essential for optimum growth and development and a constituent of various organic compounds such as protein, nucleic acid, and hence part of protoplasm. It encourages rapid vegetative growth and regulates the utilization of phosphorus and potassium uptake by the plant from the soil (Tyoakoso et al., 2019). Some agricultural practices which involve the use of agrochemicals are very harmful to human health due to their residue on food crops being consumed by humans (Enagbonma and Babalola, 2019). The application of *N* fertilizer at the seedling stage usually increases the growth and yield of Bambara groundnut (Hasan et al., 2018). However, its negative effect on the environment includes soil acidification and compaction (Guo et al., 2010; Cai et al., 2015), eutrophication of surface water (Zhang et al., 2011), accelerating global warming by greenhouse gases emission (Battye et al., 2017), and decreasing crop leaf area, photosynthetic assimilation, and seed growth.

Although urea is the most suitable source of *N* fertilizer recommended for Bambara groundnut (Hasan et al., 2019), leguminous crops usually live in association with Rhizobium bacteria which helps to improve the soil status (Sarr et al., 2016). Therefore, successful rhizobial inoculation in the field is usually measured by the extent of nodulation, nitrogen fixation (N_2), plant growth, and grain yield when compared with the uninoculated control (Kyei-Boahen et al., 2017). Also, bacteria play a vital role in the decomposition of organic matter, transformation of nutrients, and regulation of soil productivity for plant growth and development (Oburger and Schmidt, 2016). The inoculation of bacterial strains to enhance the productivity of leguminous crops commenced with discovering the beneficial effects of the plant growth-promoting rhizobacteria and the method to use it by applying it to the seed. The most common methods include seed treatments, soil treatments, and root dipping of the bacteria in suspension before transplanting (Mahmood et al., 2016; Ikenganyia et al., 2017). Farmers usually experience difficulty procuring inorganic *N* fertilizer due to its high cost, and those who can afford it typically apply it below the manufacturer-recommended levels. Therefore, the aim of this study was to compare the effect of the inoculation of *B. japonicum* strains and *N* fertilizer application on the field performance of Bambara groundnut accessions.

MATERIALS AND METHODS

An experiment was carried out in 2019 and 2020 during the rainy season between August and December in two different geographical locations, Ibadan (Ib) and Ikenne, in Nigeria. Ikenne can be characterized as a tropical savanna, with a latitude (lat) of 6° 51′ 56″ *N* and a longitude (long) of 3° 42′ 54″ E, while Ibadan can be characterized as subhumid, with a lat of 7° 22’ 30 *N* and a long of 3° 45’ 54 E.

The experiment was arranged in a randomized complete block design (RCBD) and was replicated three times in the field, with each block representing each replicate. The first block had accession seeds coated with the bacterial strains. The 10 accessions of Bambara groundnut used in this study were randomly selected from the gene bank of the International Institute of Tropical Agriculture (IITA), while the *B. japonicum* strains were type strains readily available at the soil microbiology unit at IITA. The randomly selected 10 accessions from the gene bank were as follows: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475, and TVSu-305. Six sets of seeds of each accession were coated with each of the four *Bradyrhizobium japonicum* strains (FA3, RACA6, USDA110, and IRJ2180A) and with gum Arabic 20% (w/v) to attach the strain to the seed before planting. The seeds were carefully dipped into the mixture of gum Arabic and *B. japonicum* strains, carefully stirred and allowed to stick firmly to the seeds, carefully spread and labeled to prevent mix up and allowed to dry properly before the commencement of the planting operation. The seeds coated with the bacterial strains were sown into a 2-m plot with 25 cm between each plot and 1 m between each replicate block.

In the second block, *N* fertilizer (20kg/ha) was applied to uninoculated seedlings at 2 WAP (Tyoakoso et al., 2019). The third block had the control which had no bacterial strain-coated seeds or seedlings administered with urea.

Each block was identified with a plastic peg showing the accession number and bacteria strains and *N* fertilizer application. Data were collected on the percentage of germination after planting seeds at 2 WAP, on growth traits at 7 and 12 WAP in the flowering stage, and on yield and yield components at harvest in both locations and seasons.

Land Preparation

The field was prepared mechanically using a tractor-driven plow in both locations. It was carefully pulverized and harrowed to remove debris. Afterward, plots of 2 m with spacing of 25 cm between the plot and 1 m between the blocks were prepared.

Gum Arabic Preparation

A total of 20 g of gum Arabic was suspended in 100 ml of hot sterile water (22°C) to enable the gum Arabic to dissolve instantly. It was then mixed with 10 g of peat inoculant of *B. japonicum* strains, which was eventually used to inoculate 1 kg of the accessions of Bambara groundnut (Ogbuehi, 2020).

Planting Operation

The seeds of the 10 accessions were coated with the *Bradyrhizobium japonicum* strains and allowed to dry before planting to ensure the strains were firmly coated on the seeds. Six coated seeds were sown on each plot with one seed per stand and were replicated three times in the field and prepared for the treatment (bacteria and *N* fertilizer) and uninoculated control.

Fertilizer Application

Urea was applied to seedlings at 2 WAP to compare the effect of the nitrogen-fixing bacteria, *B. japonicum*, strains with the urea. Approximately 20 kg ha^{-1} of urea was applied to each accession of the Bambara groundnut (Tyoakoso et al., 2019) and planted separately in the field without bacteria inoculation.

Weeding Operation

Weeding of the plot was carried out using chemical herbicides before their pre-emergence (lifeline 3 L/ha and metaforce 2 L/ha according to manufacturers' instructions) and manually using a hoe to weed at 6 WAP and 10 WAP to ensure optimum yield.

Insecticide Application

Spraying of insecticides was carried out post-emergence using Karate and Termex 1 L/ha according to manufacturers' instructions in both locations and seasons to control whiteflies sucking the leaves of Bambara groundnut.

Data Collection

Data on percentage germination and growth parameters at 7 WAP and 12 WAP, which include plant height (cm), number of leaves, number of stems, plant spread (cm), number of branches, leaf area, terminal leaf length (cm), terminal leaf width (cm), chlorophyll content of leaf (mg/L), number of days to 1st flowering and to 50% flowering, and fresh and dry shoot at 50% flowering and at harvest, were collected in both locations (Ib and Ik). Additionally, the number of nodules, the weight of nodules (g), and the size and shape of nodules were also recorded. At harvest, the number of pods, the weight of pods (g), pod length (g), pod width (g), seed length (g), seed width (g), yield/plot (g), yield/ha (kg/ha), and weight of 100 seeds were further recorded.

Data Analysis

Data collected were subjected to the analysis of variance (ANOVA) using a four-way ANOVA, and DMRT at $p > 0.05$ was used for mean comparison.

Soil Analysis

The pH of the soil in the Ikenne field was acidic in nature with values of 4.46 ± 0.08 and 4.91 ± 0.09 in both seasons, while, in Ibadan, the pH was neutral with values of 6.84 ± 0.1 and 7.13 ± 0.11 in both seasons. The percentage of organic carbon was analyzed following the procedure of Walkley and Black (1934). It ranged from 0.29 to 0.40 in both locations and seasons, which showed that the soil used was normal. The percentages of nitrogen (N), phosphorus (P), and potassium (K) ranged from 0.04 to 0.15, 2.55 to 52.84, and 0.22 to 0.56, respectively. The percentage of *N* was determined using the Kjeldahl method (Mulvaney and Page, 1982). The available P (ppm) was determined using Bray's method. A higher percentage of P was recorded in the soil in both locations and seasons (**Table 1**). The exchangeable potassium, sodium, and calcium were determined by ammonium acetate (Black et al., 1965). The percentage of K present in the soil in both locations and seasons showed that it was available in moderate quantities. The calcium (Ca, Cmol/kg) in the soil from the Ibadan field in 2019 had a value of 1.216 ± 0.10, which is, according to Walkley and Black (1934), considered normal. However, the soil in Ikenne for the 2019 season had a very high value of 3.53 ± 2.50, which is considered abnormal. Higher magnesium (Mg, Cmol/kg) values were obtained in Ikenne, 0.80 ± 0.37 and 0.404 ± 0.02 for 2019 and 2020, respectively, while lower quantities of Mg were obtained in Ibadan, 0.075 ± 0.01 and 0.158 ± 0.05 for 2019 and 2020, respectively (**Table 1**). The particle size analysis was determined by the hydrometer method. The texture class of the soil on the field showed that it was loamy sand because it ranged from 76.00 to 83.00% sand, 8.00 to 19.00% clay, and 3.00 to 10.00% silt. The mycorrhizal spore count was low in Ibadan with mean values of 382 and 392 in both 2019 and 2020, respectively, compared to that in Ikenne, which had mean values of 412 and 432 for 2019 and 2020, respectively (**Table 1**).

Crop Establishment

The coated Bambara groundnut seeds after planting in both locations and seasons developed into seedlings with multiple stems and branches and increased in growth every week. During flowering, the increase in the number of flowers was recorded from the number of days to first flowering to the number of days to 50% flowering. The space occupied by the plant, its canopy spread until when its growth ceased, and the commencement of reproductive stages (pod formation) were also calculated.

TABLE 1 | Physiochemical and biological properties of soil used for the studies.

Planting season properties/location	2019 Ikenne	2020 Ikenne	2019 Ibadan	2020 Ibadan
pH (H_2O) 1:1	4.46 ± 0.08	4.91 ± 0.09	6.84 ± 0.1	7.13 ± 0.11
N (%)	0.073 ± 0.02	0.121 ± 0.01	0.150 ± 0.013	0.119 ± 0.02
OC (%)	0.329 ± 0.07	0.297 ± 0.01	0.407 ± 0.10	0.336 ± 0.06
Bray *P* (ppm)	13.23 ± 4.26	22.46 ± 3.43	15.352 ± 3.74	52.84 ± 6.67
Sand (%)	76.00 ± 1.16	76.00 ± 1.16	83.00 ± 1.16	82.00 ± 0.00
Clay (%)	16.00 ± 1.16	20.00 ± 2.00	10.00 ± 0.00	8.00 ± 0.00
Silt (%)	6.00 ± 1.15	3.00 ± 1.15	7.00 ± 1.15	10.00 ± 0.0
Ca (Cmol/kg)	3.530 ± 2.50	1.505 ± 0.24	1.216 ± 0.10	1.101 ± 0.30
Mg (Cmol/kg)	0.80 ± 0.37	0.404 ± 0.02	0.075 ± 0.01	0.158 ± 0.05
K (Cmol/kg)	0.560 ± 0.24	0.242 ± 0.06	0.137 ± 0.02	0.201 ± 0.02
Na (Cmol/kg)	0.076 ± 0.01	0.082 ± 0.00	0.055 ± 0.02	0.057 ± 0.01
ECEC (Cmol/kg)	4.96 ± 1.56	2.23 ± 0.57	1.29 ± 0.56	1.47 ± 0.48
Zn (ppm)	1.964 ± 1.67	1.196 ± 0.19	1.19 ± 0.10	0.66 ± 0.04
Cu (ppm)	1.167 ± 0.62	2.045 ± 0.19	0.86 ± 0.11	0.97 ± 0.22
Mn (ppm)	12.15 ± 10.5	116.7 ± 3.31	244.20 ± 18.4	383.6 ± 33.4
Fe (ppm)	25.58 ± 7.47	88.29 ± 4.08	133.33 ± 3.33	114.4 ± 3.85
Glomus	106	117	135	152
Acaulospora	192	185	202	208
Enthrophospora	25	28	34	32
Spore/100 gdwt	380	392	412	432
Soil textural class	Loamy sand	Loamy sand	Loamy sand	Loamy sand

RESULTS

Interplay Between *B. japonicum* Strains and *N* Fertilizer Application on Growth Traits, Flowering Stages, and Biomass Yield of Bambara Groundnut Accessions

Table 2 shows the comparison of the effect of *B. japonicum* strains and urea (46% *N* fertilizer) application on growth traits and flowering stages of inoculated accessions of Bambara groundnut at 7 WAP in Ibadan and Ikenne in the first season. As seen in **Table 2**, the IRJ2180A bacterial strain had a significantly enhanced germination percentage with a mean value of 67.32% compared to other inoculated strains and *N* fertilizers. Bacterial strains, FA3 and IRJ2180A, significantly enhanced plant height of the accessions with mean values of 19.88 and 19.89 cm, respectively, compared to all other accessions, whether inoculated with *N* fertilizer or uninoculated control. On the other hand, *N* fertilizer significantly enhanced the number of leaves and number of stems compared to other inoculated accessions and uninoculated control. However, FA3-inoculated accessions and uninoculated control showed a significantly enhanced number of days to first flowering compared to other accessions inoculated with USDA110, IRJ2180A, and RACA6 strains or applied with *N* fertilizer. Significant differences were, however, observed in the number of branches among accessions inoculated with FA3 and RACA6 strains, with *N* fertilizer applied, compared to USDA110 and IRJ2180A inoculated accessions and the uninoculated control.

Impact of *B. japonicum* Strains and *N* Fertilizer on Growth Traits at 7 WAP and 12 WAP of Bambara Groundnut Accessions

The comparison of the effect of *B. japonicum* strains and urea on the growth traits and biomass yield of Bambara groundnut accessions at 12 WAP in both locations in the first season revealed that the FA3 strain significantly enhanced plant height, canopy spread, petiole length, terminal leaf width, number of leaves, number of stems, and peduncle length compared to accessions inoculated with other bacterial strains or when *N* fertilizer was applied, or under uninoculated control (**Table 3**). Similarly, the IRJ2180A strain significantly enhanced the terminal leaf length of the accessions of Bambara groundnut more than other bacterial strains, or *N* fertilizer, and the uninoculated control. Furthermore, the number of branches was significantly enhanced by the inoculation with FA3, USDA110, and RACA6 compared to IRJ2180A inoculation, *N* fertilizer application, or uninoculated control. Similarly, RACA6 significantly enhanced the leaf area compared to other inoculated strains and application of *N* fertilizer. However, there were no significant differences observed in chlorophyll content of leaf, fresh and dry shoots at flowering and fresh and dry roots at flowering.

The comparison of the effect of bacterial strains and urea on growth traits at flowering at 7 WAP in both locations in the second season is shown in **Table 4**. At 7 WAP there were no significant differences in germination percentage, petiole length, chlorophyll content of leaf, and peduncle length

TABLE 2 | Effect of *N* fertilizer and *B. japonicum* strains on the percentage of germination at 2 WAP, growth traits of accessions of Bambara groundnut at 7 WAP, and flowering in the first season in Ibadan and Ikenne 2019.

Treatment	% GM	PLH (cm)	NOL	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	GH	D50% Flw	D1st Flw	PL (cm)	PEL (cm)	CS (cm)	LA (cm2)
FA3	56.17^{d}	19.89^{a}	118.97^{ab}	35.41^{a}	39.83^{ab}	5.28^{a}	2.17^{a}	43.52^{a}	2.08^{a}	54.97^{bc}	41.63^{a}	7.74^{a}	1.77^{a}	10.44^{a}	11.79^{a}
USDA110	58.33^{cd}	19.66^{ab}	111.20^{b}	32.22^{b}	37.78bb	5.51^{a}	2.13^{a}	44.86^{a}	2.12^{a}	55.43^{ab}	42.54^{b}	7.91^{a}	1.67^{a}	10.70^{a}	12.38^{a}
N	63.75^{ab}	19.53^{ab}	129.36^{a}	37.89^{a}	43.52^{a}	5.37^{a}	2.13^{a}	44.85^{a}	2.14^{a}	54.71^{bc}	42.14^{b}	7.72^{a}	1.72^{a}	10.52^{a}	11.74^{a}
IRJ2180A	67.32^{a}	19.88^{a}	120.93^{ab}	33.18^{b}	39.46^{ab}	5.26^{a}	2.28^{a}	44.50^{a}	2.11^{a}	54.71^{bc}	41.82^{b}	7.61^{a}	1.82^{a}	10.48^{a}	12.24^{a}
RACA6	62.32^{bc}	19.69^{ab}	123.44^{ab}	36.14^{a}	42.04^{ab}	5.35^{a}	2.22^{a}	42.41^{a}	2.12^{a}	54.49^{c}	41.86^{b}	8.03^{a}	1.73^{a}	10.16^{a}	12.13^{a}
CONTROL	54.17^{d}	18.29^{b}	109.75^{b}	32.57^{b}	37.89^{b}	5.23^{a}	2.21^{a}	40.75^{a}	2.09^{a}	55.78^{a}	43.55^{a}	6.63^{b}	1.76^{a}	10.09^{a}	12.10^{a}

Means with the same letter are not significantly different at a $P > 0.05$ level of probability using the Duncan multiple range test (DMRT).%GM, percentage of germination; PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll; GH, growth habit; D50% Flw, days to 50% flowering; D1st Flw, days to first flowering; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 3 | Effect of *N* fertilizer and *B. japonicum* strains on growth trait and biomass yield of accessions of B. groundnut at 12 WAP in Ibadan and Ikenne in the first season, 2019.

Treatment	PLH (cm)	CS (cm)	PEL (cm)	TLL (cm)	TLW (cm)	NOB	NOL	NOS	LA (cm^2)	CPYL (mg/L)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.61^{a}	12.21^{a}	1.76^{a}	5.98^{b}	2.55^{a}	62.77^{a}	215.88^{a}	72.68^{a}	15.39^{b}	35.74^{a}	14.12^{a}	33.05^{a}	12.22^{a}	10.07^{a}	3.39^{a}
USDA110	21.68^{bc}	11.24^{b}	1.61^{b}	5.79^{b}	2.35^{b}	59.03^{a}	195.00^{b}	64.85^{b}	13.74^{b}	35.76^{a}	10.94^{b}	28.42^{a}	10.82^{a}	9.39^{a}	3.39^{a}
N	21.64^{bc}	11.39^{ab}	1.69^{ab}	5.90^{b}	2.46^{ab}	54.30^{b}	162.33^{b}	62.00^{b}	14.59^{b}	37.29^{a}	10.35^{b}	30.41^{a}	10.76^{a}	9.86^{a}	3.33^{a}
IRJ2180A	22.42^{ab}	11.53^{ab}	1.59^{b}	11.12^{a}	2.45^{ab}	56.59^{b}	190.77^{b}	63.59^{b}	14.89^{b}	36.40^{a}	10.73^{b}	31.59^{a}	11.71^{a}	9.69^{a}	3.48^{a}
RACA6	21.69^{bc}	11.62^{ab}	1.65^{ab}	5.69^{b}	2.47^{ab}	57.08^{a}	188.26^{b}	64.54^{b}	21.34^{a}	36.21^{a}	10.83^{b}	34.79^{a}	12.77^{a}	12.12^{a}	4.14^{a}
CONTROL	20.90^{c}	11.14^{b}	1.64^{ab}	5.84^{b}	2.44^{ab}	49.21^{c}	182.71^{b}	56.48^{c}	14.44^{b}	33.97^{a}	9.74^{b}	24.77^{b}	10.91^{a}	9.51^{a}	3.76^{a}

Means with the same letter are not significantly different at $P > 0.05$ level of probability using the Duncan multiple range test (Dmrt). PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting. FSF, fresh shoot at flowering; DSF, dry shoot at flowering; FRF, fresh root at flowering; DRF, dry root at flowering.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

among strains inoculated on accessions, application of *N* fertilizer, and uninoculated control. Similarly, there were no significant differences in the number of leaves, number of branches, and number of stems in all accessions barring those that were treated with RACA6 or urea. Plant height was significantly enhanced by FA3 and USDA110 strains compared to those inoculated with IRJ2180A and RACA6. Accessions with *N* fertilizer application had the lowest mean value in plant height. Also, terminal leaf length was significantly enhanced with the inoculation of FA3 and USDA110 strains compared to that of IRJ2180A and RACA6 strains and uninoculated control. On the other hand, *N* fertilizer-applied accessions recorded the lowest terminal leaf length. It was further noted that terminal leaf width and days to 50% flowering significantly decreased with *N* fertilizer application compared to the bacterial strain-inoculated accessions or the uninoculated control.

Impact of *B. japonicum* Strain Inoculation and *N* Fertilizer Application on Growth Traits, Flowering, and Biomass Yield at 7 WAP and 12 WAP

Table 4 shows that FA3 significantly enhanced the number of days to the first flowering of accessions compared to other strains or uninoculated control; *N* fertilizer had the lowest mean value. Similarly, analyzing the growth traits and biomass yield at 12 WAP in both locations in the first season revealed that FA3 significantly enhanced plant height, petiole length, terminal leaf width, number of branches, number of leaves, chlorophyll content of leaf, and peduncle length more than other strains (**Table 5**); *N* fertilizer recorded the lowest mean value on the growth traits. Also, RACA6 significantly enhanced biomass yield (dry shoot at flowering, and fresh and dry roots at flowering) compared to other strains, or when *N* fertilizer was applied, or uninoculated control. The FA3 strain also significantly enhanced plant height while *N* fertilizer had the lowest mean value (**Table 6**). It was further revealed that USDA110 significantly enhanced terminal leaf length compared to other strains, when *N* fertilizer was applied, or uninoculated control. **Table 6** demonstrates that, though there were no significant differences in terminal leaf length between inoculated accessions and uninoculated control in both seasons, there was a significant difference between the inoculated strains and *N* fertilizer-applied accessions. The peduncle length was significantly enhanced among FA3 and USDA110-inoculated accessions in both seasons, and the leaf area was significantly enhanced by USDA110 compared to *N* fertilizer. A comparison of the effect of the bacterial strains and *N* fertilizer on growth traits and biomass yield at 12 WAP in both locations and seasons shows no significant differences. However, as seen in **Table 7**, FA3 significantly enhanced all growth traits at 12 WAP except for terminal leaf length and leaf areas, which were significantly enhanced by IRJ2180A and RACA6.

Impact of *B. japonicum* Strains and *N* Fertilizer Application on the Yield and Yield Components of Bambara Groundnut Accessions

Significant differences were recorded in the seed length, yield/plot, yield/ha, pod/plant, pod/plot, weight of pod/plot, seed/plot, seed weight/plot, and shelling percentage at harvest among the strains inoculated, the *N* fertilizer applied, and uninoculated control in both locations in the first season (**Table 8**). The application of nitrogen fertilizer and inoculation of IRJ2180A were found to significantly enhance yield/plot at harvest. On the other hand, RACA6 strains significantly enhanced yield/ha, pod/plant, weight of pod/plot, and seed weight/plot at harvest compared to other strains, *N* fertilizer application, and uninoculated control in the first season. Similarly, in both locations in the second season, significant differences were recorded in the pod width, seed width, 100 seed weight, yield/plot, yield/ha, pod/plant, pod/plot, weight of pod/plot, seed/plant, seed/plot, seed weight/plot, and shelling percentage at harvest between accessions inoculated with bacterial strains, application of *N* fertilizer, and uninoculated control. While inoculation with FA3 strain was found to significantly enhance pod width, seed width, 100 seed weight, pod/plant, and seed/plant at harvest compared to inoculations with other strains and application with *N* fertilizer in the second season. Yield/plot, yield/ha, pod/plot, weight of pod/plot, and seed weight/plot at harvest were found to be significantly enhanced when inoculated with USDA110 strains (**Table 9**). Significant differences were also recorded in all other yield and yield components after harvest between accessions inoculated with bacterial strains, *N* fertilizer application, and uninoculated control in both locations in both seasons. Interestingly, pod width, seed length, seed width, and weight of pod/plot at harvest were found to be significantly enhanced in the uninoculated control compared to inoculated accessions or those applied with *N* fertilizer (**Table 10**). The FA3 strain significantly enhanced 100 seed weight, seed/plant, and seed weight/plot of accessions at harvest in both seasons, while the RACA6 strain significantly enhanced yield/plot and yield/ha. Among bacterial strains, FA3, RACA6, and USDA110 significantly enhanced pod/plant and seed/plot of the accessions. **Table 11** provides a cumulative overview of the significant differences recorded among accessions inoculated with different *B. japonicum* strains in different seasons, locations, and its yield and yield components.

DISCUSSION

Response of Bambara Groundnut to Inoculation and *N* Fertilizer Application

The differences in the impact recorded in the growth and yield of the Bambara groundnut accessions due to the inoculation of *B. japonicum* strains coated to the seeds before planting in both locations and seasons are similar to the result obtained by Sanginga et al. (1996) and Houngnandan et al. (2000), which

TABLE 4 | Effect of *N* fertilizer and *B. japonicum* strains on the percentage of germination at 2 WAP, growth traits of accessions of Bambara groundnut at 7 WAP, and flowering in Ibadan and Ikenne in the second season, 2020.

Treatment	% GM	PEL (cm)	PLH (cm)	NOL	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	D50% Flw	D1st Flw	PL (cm)	LA (cm^2)
FA3	78.17^{a}	1.43^{a}	19.62^{a}	116.10^{a}	38.45^{a}	38.92^{a}	6.06^{a}	2.06^{a}	42.37^{a}	54.93^{a}	45.83^{a}	7.99^{a}	12.56^{ab}
USDA110	80.38^{a}	1.42^{a}	19.63^{a}	118.32^{a}	39.13^{a}	39.68^{a}	5.99^{a}	2.08^{a}	43.73^{a}	49.03^{b}	44.18^{ab}	7.16^{a}	13.02^{a}
N	70.75^{a}	1.57^{a}	17.18^{b}	97.78^{b}	32.38^{b}	32.83^{b}	5.18^{b}	1.75^{b}	39.67^{a}	44.82^{c}	38.83^{c}	6.78^{a}	10.75^{b}
IRJ2180A	78.17^{a}	1.39^{a}	18.79^{ab}	114.88^{a}	37.04^{a}	38.30^{ab}	5.76^{ab}	2.04^{a}	43.78^{a}	52.95^{ab}	44.28^{ab}	6.72^{a}	12.24^{ab}
RACA6	77.00^{a}	1.41^{a}	18.01^{ab}	110.20^{ab}	36.30^{ab}	36.57^{ab}	5.48^{ab}	2.15^{a}	40.29^{a}	51.67^{ab}	41.63^{bc}	6.37^{a}	12.89^{a}
CONTROL	78.55^{a}	1.80^{a}	19.97^{a}	119.82^{a}	39.33^{a}	39.73^{a}	5.74^{ab}	2.13^{a}	43.80^{a}	54.87^{a}	43.27^{ab}	6.45^{a}	12.93^{a}

Means with the same letter are not significantly different at $P < 0.05$ level of probability using the Duncan multiple range test (DMRT).%GM, percentage of germination; PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll content of leaf; GH, growth habit; D50% Flw, days to 50% flowering; D1st Flw, days to first flowering; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 5 | Effect of *N* fertilizer and *B. japonicum* strains on growth traits and biomass yield of accessions of Bambara groundnut at 12 WAP in Ibadan and Ikenne in the second season, 2020.

Treatment	PLH (cm)	PEL (cm)	TLL (cm)	TLW (cm)	NOB	NOL	NOS	LA (cm^2)	CPYL (mg/L)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.80^{a}	1.69^{a}	6.02^{b}	2.56^{a}	62.70^{a}	202.62^{a}	67.53^{a}	33.92^{a}	28.15^{a}	11.26^{a}	20.38^{a}	6.76^{ab}	6.21^{ab}	1.25^{ab}
USDA110	19.51^{c}	1.43^{bc}	5.29^{cd}	2.17^{c}	52.28^{b}	166.45^{b}	58.97^{a}	31.95^{a}	12.59^{d}	9.22^{ab}	19.42^{a}	6.73^{ab}	6.42^{ab}	1.16^{ab}
N	17.79^{d}	1.36^{c}	4.86^{d}	2.12^{c}	45.72^{c}	143.12^{c}	49.31^{b}	28.15^{b}	12.41^{d}	8.39^{b}	18.76^{a}	5.97^{ab}	6.08^{ab}	1.00^{ab}
IRJ2180A	21.71^{ab}	1.56^{ab}	10.06^{a}	2.37^{b}	54.05^{b}	171.98^{b}	63.63^{a}	30.99^{ab}	14.24^{bc}	9.81^{ab}	19.48^{a}	6.83^{ab}	5.98^{b}	1.28^{ab}
RACA6	20.95^{bc}	1.55^{ab}	5.42^{c}	2.37^{b}	57.05^{ab}	177.17^{b}	60.98^{a}	32.29^{a}	13.91^{c}	9.47^{ab}	23.19^{a}	7.55^{a}	8.08^{a}	1.37^{a}
CONTROL	21.25^{ab}	1.63^{a}	5.96^{a}	2.49^{ab}	55.15b	172.55^{b}	59.58^{a}	34.22^{a}	15.23^{a}	9.22^{ab}	17.34^{a}	5.13^{b}	6.66^{ab}	0.85^{b}

Means with the same letter are not significantly different at $P < 0.05$ level of probability using the Duncan multiple range test (DMRT). PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll content of leaf; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting. FSF, fresh shoot at flowering; DSF, dry shoot at flowering; FRF, fresh root at flowering; DRF, dry root at flowering.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 6 | Effect of *N* fertilizer and *B. japonicum* strains on the percentage of germination at 2 WAP, growth traits of accessions of Bambara groundnut at 7 WAP, and flowering in Ibadan and Ikenne during both seasons.

Treatment	%GM	PLH (cm)	NOL (cm)	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	D1st Flw	D50% Flw	PL (cm)	PEL (cm)	CS (cm)	LA (cm^2)
FA3	67.17^{a}	19.59^{a}	116.54^{a}	36.63^{a}	39.04^{a}	5.62^{ab}	2.09^{a}	42.22^{a}	43.38^{a}	54.95^{a}	7.80^{a}	1.58^{a}	10.74^{a}	12.08^{ab}
USDA110	69.36^{a}	19.47^{ab}	113.83^{a}	35.41^{a}	38.42^{a}	5.70^{a}	2.09^{a}	43.54^{a}	43.00^{ab}	51.31^{b}	7.47^{a}	1.53^{a}	10.65^{ab}	12.59^{a}
N	65.13^{a}	18.03^{b}	111.42^{a}	34.51^{a}	37.89^{a}	5.18^{b}	1.91^{b}	41.14^{a}	39.78^{c}	49.73^{c}	7.12^{ab}	1.62^{a}	9.72^{c}	11.05^{b}
IRJ2180A	70.50^{a}	18.84^{ab}	114.88^{a}	34.28^{a}	37.89^{a}	5.38^{ab}	2.10^{a}	43.03^{a}	42.00^{abc}	49.73^{b}	6.98^{ab}	1.56^{a}	10.37^{abc}	11.93^{ab}
RACA6	67.58^{a}	18.36^{ab}	113.73^{a}	35.32^{a}	38.25^{a}	5.28^{ab}	2.13^{a}	40.29^{a}	40.70^{bc}	51.72^{b}	6.99^{ab}	1.52^{a}	9.91^{bc}	12.20^{ab}
CONTROL	66.36^{a}	18.52^{ab}	111.13^{a}	34.87^{a}	37.55^{a}	5.31^{ab}	2.09^{a}	40.92^{a}	41.96^{abc}	55.33^{a}	6.32^{b}	1.72^{a}	10.05^{abc}	12.11^{ab}

Means with the same letter are not significantly different at $P < 0.05$ level of probability using the Duncan multiple range test (DMRT).%GM, percentage of germination; PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll content of leaf; GH, growth habit; D50% Flw, days to 50% flowering; D1st Flw, days to first flowering; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 7 | Effect of *N* fertilizer and *B. japonicum* strains on growth traits and biomass yield of accessions of Bambara groundnut at 12 WAP in Ibadan and Ikenne during both seasons.

Treatment	PLH (cm)	CS (cm)	PEL (cm)	TLL (cm)	TLW (cm)	NOB	NOL	NOS	LA (cm^2)	CPYL (SPAD)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.71^{a}	12.21^{a}	1.73^{a}	6.00^{b}	2.56^{a}	62.73^{a}	209.25^{a}	70.10^{a}	15.74^{ab}	34.53^{a}	12.69^{a}	21.68^{a}	6.31^{a}	8.38^{a}	1.90^{a}
USDA110	20.23^{b}	10.37^{bc}	1.49^{c}	5.44^{b}	2.22^{c}	54.68^{b}	177.48^{b}	60.83^{b}	12.94^{b}	33.26^{abc}	9.89^{b}	19.53^{a}	6.46^{a}	7.73^{a}	1.72^{a}
N	18.99^{c}	10.37^{c}	1.47^{c}	5.18^{b}	2.20^{c}	48.20^{c}	156.83^{c}	53.59^{c}	13.02^{b}	31.48^{bc}	9.025^{b}	18.29^{a}	5.94^{a}	7.54^{a}	1.80^{a}
IRJ2180A	20.38^{b}	10.46^{bc}	1.46^{c}	9.75^{a}	2.22^{c}	51.08^{bc}	167.07^{bc}	58.84^{bc}	13.45^{b}	30.96^{c}	9.46^{b}	19.55^{a}	6.41^{a}	7.78^{a}	1.83^{a}
RACA6	20.78^{b}	10.99^{b}	1.56^{bc}	5.42^{b}	2.36^{b}	55.64^{b}	178.01^{b}	61.15^{b}	17.09^{a}	33.35^{abc}	9.88^{b}	20.65^{a}	6.45^{a}	8.39^{a}	1.77^{a}
CONTROL	21.07^{b}	11.07^{b}	1.64^{ab}	5.90^{b}	2.46^{ab}	52.18^{bc}	167.44^{bc}	58.03^{bc}	14.83^{ab}	34.09^{ab}	9.48^{b}	19.74^{a}	5.86^{a}	8.15^{a}	1.68^{a}

Means with the same letter are not significantly different at $P < 0.05$ level of probability using the Duncan multiple range test (DMRT). PLH, plant height; NOL, number of leaves; NOS, number of stems; NOB, number of branches; TLL, terminal leaf length; TLW, terminal leaf width; CPYL, chlorophyll content of leaf; PL, peduncle length; PEL, petiole length; CS, canopy spread; LA, leaf area; WAP, weeks after planting. FSF, fresh shoot at flowering; DSF, dry shoot at flowering; FRF, fresh root at flowering; DRF, dry root at flowering.

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 8 | Effect of *N* fertilizer and *B. japonicum* strains on yield and yield components of Bambara groundnut accessions at harvest in Ibadan and Ikenne in the first season, 2019.

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield /Plot (g)	yield/ha (kg)	Pod /Plant	Pod/plot (g)	Wgt of pod/plot (g)	Seed/plant	Seed/ plot	Seed wgt/plot (g)	Shelling %
FA3	17.12^{a}	11.49^{a}	12.64^{b}	9.23^{a}	55.79^{a}	70.25^{ab}	1,401.8^{b}	29.39^{b}	100.25^{ab}	73.24^{ab}	27.23^{a}	93.89^{a}	41.61^{ab}	41.42^{ab}
USDA110	17.28^{a}	11.69^{a}	12.38^{b}	9.63^{a}	53.32^{a}	63.54^{b}	1,367.7^{c}	27.65^{b}	95.20^{c}	67.62^{b}	27.01^{a}	96.13^{a}	38.14^{b}	44.49^{a}
N	17.85^{a}	12.02^{a}	13.59^{ab}	9.63^{a}	55.29^{a}	75.61^{a}	1,394.9^{c}	31.26^{a}	109.39^{a}	73.71^{ab}	25.29^{a}	97.47^{a}	42.86^{ab}	42.86^{ab}
IRJ2180A	17.11^{a}	11.93^{a}	12.47^{b}	9.53^{a}	58.19^{a}	75.05^{a}	1,459.9b	28.80^{b}	95.43^{c}	74.44^{ab}	27.61^{a}	88.68^{b}	42.38^{ab}	39.48^{b}
RACA6	16.89^{a}	11.78^{a}	13.39^{ab}	9.72^{a}	54.31^{a}	81.19^{ab}	1,582.7^{a}	30.53^{a}	102.26^{ab}	86.08^{a}	27.74^{a}	96.72^{a}	44.66^{a}	45.38^{a}
CONTROL	17.34^{a}	11.76^{a}	14.78^{a}	9.85^{a}	55.19^{a}	64.41^{b}	1,201.6^{d}	21.79^{c}	80.35^{d}	78.37^{ab}	28.47^{a}	70.82^{c}	38.99^{b}	41.89^{ab}

Means with the same letter are not significantly different at a $P < 0.05$ level of probability using the Duncan multiple range test (DMRT).

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 9 | Effect of *N* fertilizer and *B. japonicum* strains on yield and yield components of Bambara groundnut accessions at harvest in Ibadan and Ikenne in the second season, 2020.

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield/plot (g)	yield/ha (kg)	Pod /Plant	Pod/plot (g)	Wgt of spod/plot (g)	Seed/ plant	Seed/ plot	Seed wgt/plot (g)	Shelling %
FA3	10.29^{a}	7.24^{a}	6.79^{a}	5.07^{a}	60.70^{a}	34.71^{ab}	125.04^{ab}	16.06^{a}	42.17^{ab}	32.02^{ab}	16.03^{a}	41.11^{a}	24.39^{ab}	26.79^{a}
USDA110	10.80^{a}	7.36^{ab}	6.66^{a}	5.01^{ab}	56.51^{ab}	43.85^{a}	130.59^{a}	12.88^{abc}	44.08^{a}	34.51^{a}	14.81^{ab}	42.91^{a}	27.10^{a}	23.20^{a}
N	9.74^{a}	6.30^{b}	5.78^{a}	4.23^{b}	45.05^{b}	25.04^{b}	118.58^{b}	9.26^{c}	28.65^{b}	22.23^{c}	10.97^{c}	27.48^{b}	16.01^{c}	24.51^{ab}
IRJ2180A	9.923^{a}	7.08^{b}	6.23^{a}	4.74^{ab}	54.84^{ab}	33.92^{ab}	106.56^{b}	12.15^{bc}	40.04^{ab}	28.29^{b}	12.99^{b}	39.26^{ab}	19.86^{bc}	23.24^{a}
RACA6	9.74^{a}	7.00^{b}	6.33^{a}	4.82^{ab}	56.46^{ab}	31.29^{b}	103.39^{b}	12.14^{bc}	37.12^{ab}	27.67^{b}	12.96^{b}	35.87^{ab}	20.49^{abc}	22.04^{b}
CONTROL	10.29^{a}	9.36^{a}	6.76^{a}	4.91^{ab}	42.09^{b}	26.69^{b}	80.47^{c}	14.19^{ab}	24.15^{c}	31.29^{ab}	6.50^{d}	20.62^{c}	23.61^{ab}	24.00^{ab}

Means with the same letter are not significantly different at a $P < 0.05$ level of probability using the Duncan multiple range test (DMRT).

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 10 | Effect of *N* fertilizer and *B. japonicum* strains on yield and yield components of Bambara groundnut accessions at harvest in Ibadan and Ikenne during both seasons.

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield/plot (g)	yield/ha (kg)	Pod /plant	Pod/plot (g)	Wgt of pod/plot (g)	Seed/ plant	Seed/plot	Seed wgt/plot (g)	Shelling %
FA3	13.27^{a}	9.08^{ab}	9.39^{ab}	6.92^{ab}	56.85^{a}	50.72^{ab}	728.39^{ab}	21.99^{a}	68.70^{a}	50.79^{ab}	20.95^{a}	65.16^{a}	31.96^{a}	33.07^{b}
USDA110	13.18^{a}	8.94^{b}	8.90^{b}	6.84^{ab}	52.25^{ab}	50.52^{ab}	680.75^{b}	18.88^{ab}	64.88^{ab}	47.68^{ab}	19.56^{ab}	64.72^{a}	30.72^{ab}	31.62^{b}
N	12.01^{a}	8.26^{b}	8.67^{b}	6.21^{b}	46.03^{b}	44.66^{b}	633.07^{c}	17.91^{b}	63.73^{ab}	42.44^{b}	17.22^{b}	55.16^{b}	26.22^{b}	30.47^{b}
IRJ2180A	12.52^{a}	8.81^{b}	8.62^{b}	6.58^{ab}	53.12^{ab}	50.11^{ab}	698.06^{b}	18.79^{ab}	62.17^{b}	47.03^{ab}	17.67^{b}	58.79^{b}	28.65^{ab}	29.11^{c}
RACA6	12.33^{a}	8.70^{b}	9.07^{ab}	6.70^{ab}	52.22^{ab}	51.51^{a}	750.72^{a}	19.56^{a}	63.73^{ab}	51.86^{ab}	18.73^{ab}	60.65^{ab}	29.97^{ab}	31.06^{b}
CONTROL	13.39^{a}	10.27^{a}	10.40^{a}	7.135^{a}	47.26^{b}	48.44^{b}	533.82^{d}	12.20^{c}	54.97^{c}	32.87^{a}	11.78^{c}	46.78^{c}	25.07^{b}	41.90^{a}

Means with the same letter are not significantly different at a $P < 0.05$ level of probability using the Duncan multiple range test (DMRT).

[a] *highly significant.*

[b] *significant.*

[c] *less significant.*

[d] *not significant.*

TABLE 11 | Analysis of accessions inoculated with *B. japonicum* strains in Ibadan and Ikenne locations during both seasons.

Source	DF	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	Wgt100 seed	Yield/plot (g)	yield/ha (kg/ha)	Pod/plot	Wgt pod/plot (g)	Seed/plot	Wgt seed/plot (g)
Accessions	9	157.89**	80.14**	66.29**	41.33**	11,572**	19,516.19**	4,516,451**	25,275.49**	23,518.6**	39,987.39**	10,103.8**
Strains	5	37.89^{ns}	53.94**	52.68**	12.15*	1,987.91*	2,327.35*	565,048.9*	3,253.76**	1,794.03*	2,373.35*	1,083.01*
Season	1	4,238.9**	1,278.9*	5,296.93*	2,696.2^{ns}	6,422.29^{ns}	201,741.8**	2.6833**	505,919.5**	272,160.4**	414,765.6**	52,271.9**
Location	1	2,966.4**	2,231.0*	4,875.5**	4,066.9^{ns}	21,817.9*	230,306.6**	1.0923**	554,858.6**	401,441.3**	510,664.9**	102,848.9**
Rep	2	201.59**	43.00^{ns}	211.75**	43.02**	574.42^{ns}	693.39^{ns}	13.4^{ns}	360.86^{ns}	356.96^{ns}	7,352.33^{ns}	160.69^{ns}
Accessions*strain	45	26.61^{ns}	25.44^{ns}	21.94^{ns}	7.71^{ns}	759.33^{ns}	1,665.43^{ns}	570,363.7^{ns}	2,968.66**	2,138.72**	5,237.34**	789.09^{ns}
Accessions*season	9	60.06*	35.15^{ns}	39.23^{ns}	17.50**	986.93^{ns}	6,257.61**	3,649,903**	20,459.03**	12,296.21**	26,870.41**	4,297.76**
Accessions*location	9	47.89^{ns}	29.73^{ns}	41.97^{ns}	10.92^{ns}	510.95^{ns}	9,438.24**	2,705,124**	15,403.54**	10,086.17**	28,445.74**	4,562.71**
Strains*season	5	19.15^{ns}	17.53^{ns}	29.23^{ns}	2.77^{ns}	591.48^{ns}	3,546.55^{ns}	473,516.6^{ns}	2,457.12^{ns}	1,628.54^{ns}	1,047.16^{ns}	900.31^{ns}
Season*location	1	51.37^{ns}	1,016.4*	125.96^{ns}	686.91*	8.01^{ns}	332,521.6**	1.1322**	569,157.2**	459,783.1**	462,964.6**	128,785.2**
Ace*stra*sea*loc	131	20.41^{ns}	18.81^{ns}	18.43^{ns}	5.83^{ns}	445.15^{ns}	2,296.82**	889,695.4^{ns}	2,822.56**	2,493.77**	5,679.69**	1,005.93**

$^{*}P < 0.01$ Significant, $^{**}P < 0.05$ highly significant, ns, not significant.

indicated that response to inoculation is likely to occur when the indigenous rhizobia population is less than 5 or 10 rhizobia cells g^{-1} soil. Furthermore, significant differences observed in the biomass yield in this study show that *B. japonicum* strains enhance the biomass yield more than the *N* fertilizer (Soe et al., 2010). This is in agreement with the research conducted by Hungria and Mendes (2015), where, compared to *N* fertilizers, bacterial strains enhanced the growth traits and the biomass yield.

The low yield recorded in the second season in both locations resulted from inadequate rainfall in Nigeria. Our study demonstrated that the growth traits, biomass yield, number of days to flowering, and yield and yield components in Bambara groundnut accessions were significantly enhanced due to the inoculation of *B. japonicum* strains compared to *N* fertilizers. This is in agreement with a study conducted by Solomon et al. (2012), which revealed that inoculation of *B. japonicum* enhanced nodulation, nitrogen fixation, and yield of soybean in Ethiopia. The results of our study will help farmers increase their profitability due to the low procuring cost of bacterial strains compared to inorganic *N* fertilizers, which are very expensive and lead to environmental contamination and adverse soil health (Igiehon and Babalola, 2018).

Role of Bacterial Strains in Improving Legume Productivity

This study revealed the importance of the inoculation of bacterial strains (*B. japonicum*) over *N* fertilizers. The Bambara groundnut accessions in both locations inoculated with four bacterial strains in this study showed a significant difference in growth, yield, and yield components compared to the *N* fertilizers applied. This indicates that the bacterial strains possessed a greater advantage over the *N* fertilizers. It is of paramount importance to introduce this technique to the end-users—the farmers—to improve their legume production and reduce the amount spent annually on inorganic fertilizers, which adversely affect the soil. The inoculation of bacterial strains to legumes is inexpensive and easily accessible.

Further Studies on Improving Legume Production Using Inoculation

In a research conducted in Chad and Cameroon, the inoculation of Bambara groundnut and groundnut in the field shows that inoculation contributed to the improvement of growth traits and biomass yield of the two legumes as well as their yield and yield components with a mean value of 63.73 kg/ha for groundnut and 72.71 kg/ha for Bambara groundnut, which is related to the results recorded in this study (Gomoung et al., 2017). In another study conducted in South Africa, inoculating *B. japonicum* and *Bacillus subtilis* strains on Bambara groundnut and cowpea revealed that co-inoculation of *B. japonicum* and *Bacillus subtilis* strains had the potential to improve the yield of both Bambara groundnut and cowpea (Nelwamondo, 2020).

CONCLUSION

The bacterial strains inoculated in this study significantly influenced the growth traits, biomass yield, and yield traits of Bambara groundnut accessions during both seasons and both locations. The results revealed that it is important to promote the use of biofertilizers over inorganic *N* fertilizers, which most farmers may not be able to afford due to their high cost, and those who can afford them usually apply *N* fertilizers below manufacturer-recommended levels. Rhizobia inoculants are readily available and farmers need to be informed about this new technique because the results of research such as this one tend to remain within the research community and do not reach the farmers. Therefore, more efforts are needed to disseminate new innovations to farmers in rural settlements and introduce this cheap and friendly technique to the poor farming community to improve the production of their legumes and increase their profit. For alternative use, FA3, USDA110, and RACA6 can be recommended for Bambara groundnut inoculations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

TB undertook conceptualization, methodology, and original draft preparation. OB and OO undertook supervision and the formal analysis and editing. MA handled project administration and funding acquisition. All the authors discussed the result and contributed to the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.