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Rhizobial biofertilizers and Bambara groundnut (*Vigna subterranea* L. Verdcourt) production: Unveiling the synergistic potential for growth and yield in Benin's challenging the sub-humid zone of Benin

Mahougnon Charlotte Carmelle Zoundji*[1,2,3], Sètondji Martin Tovizounkou[2], Tobi Moriaque Akplo[3], Arcadius Martinien Agassin Ahogle[3,4], Ibouraïman Balogoun[1,2], Felix Alladassi Kouelo[3], Appolinaire Adandonon[1,2], Pascal Houngnandan[3]

[1]*Laboratoire des Sciences Végétales, Horticoles et Forestières, Université Nationale d'Agriculture, Kétou, Bénin*
[2]*Ecole de Gestion et de Production Végétale et Semencière, Université Nationale d'Agriculture, Kétou, Bénin*
[3]*Unité de Recherche en Microbiologie du Sol, Ecologie Microbienne et Conservation des Eaux et des Sols, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Cotonou, Benin*
[4]*Department of Spatial and Environmental Planning, Kenyatta University, Nairobi, Kenya*

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Abstract

Valorization of interaction between indigenous rhizobia and local leguminous varieties is of great economic interest due to its function in enhancing agricultural productivity. The present study aims to evaluate the effect of biofertilizers based on Rhizobium on the productivity of three Bambara groundnut varieties in two semiarid Agro Ecological Zones (AEZ) of Benin (AEZ 4: West Atacora zone and AEZ 5: Cotton zone of the Centre Benin). Four inoculation treatments i.e., control uninoculated, Rhizobium strains LMSEM312, LMSEM338, USDA110 were tested on three Bambara groundnut varieties (Variety 1: *Azigokouiwéwékpè*; Variety 2: *Azigokouivovo*; Variety 3: *Azigokouiwéwékpoguè*) in a Randomized Complete Block design. Results revealed that the different Rhizobia strains improved nodulation and yield parameters of the three Bambara groundnut varieties. Among the tested strains, LMSEM338 induced the highest nodules number (30 nodules per plant) and nodule dry weight (0.34 g plant^{-1}) for the three Bambara varieties in the AEZ 4. In the AEZ 5, the best nodulation was reported on variety 1 inoculated with strain LMSEM312 (28 nodules which weighted 0.31 g). In the AEZ 4, grain yield ranged between 616.63 kg ha^{-1} (Variety 1 uninoculated) and 1918.64 kg ha^{-1} (variety 3 inoculated with strain LMSEM 338). In the AEZ 5, grain yield ranged between 518.13 kg ha^{-1} (variety 1 uninoculated) and 1596.89 kg ha^{-1} (variety 1 inoculated with strain LMSEM312). These strains could be used as a potent candidate for biofertilizer production to get sustainable yield of Bambara groundnut on marginal soils with minimum inputs.

* **Corresponding Author:** Mahougnon Charlotte Carmelle Zoundji ✉ zoundjicharlotte@gmail.com

Introduction

Bambara groundnut (*Vigna subterranea* L. Verdc.), a leguminous crop native to Africa has been cultivated over millennia and has contributed substantially to food security especially in Sub-Saharan African (SSA) (Gbaguidi *et al.*, 2015). It is a nutrient-rich crop and plays an important nutritional role for small holder farmers (Basu *et al.*, 2007; Touré *et al.*, 2013; Ibrahim *et al.*, 2018; Konate *et al.*, 2019). Moreover, it is a drought-tolerant crop and thrives in poor soil conditions, especially in low-input farming systems (Basu *et al.*, 2007; Mbaiogaou *et al.*, 2013; Mbosso *et al.*, 2020). As leguminous crops, Bambara groundnut could improves soil fertility through Biological Nitrogen Fixation (BNF), which is beneficial in intercropping and crop rotations (Mateva *et al.*, 2023). Indeed, Biological nitrogen fixation through legume and rhizobia symbiosis has been shown to increase cereal yields in rotation by 30% to 350% (Peoples and Crasswell, 1992). The N supplied through BNF accelerates plant growth, spreads fodder production over time, and leads to a significant increase in biomass (Lawlor *et al.*, 2001). Furthermore, the crop performed on marginal soils that are less suitable for producing other legume crops such as groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp.), making it a crop of choice for poor farmers under low-input farming systems (Musa *et al.*, 2016).

Despite its potential, Bambara groundnut remains an underutilized pulse legume in SSA (Gbaguidi *et al.*, 2015). Its average yield is relatively low in Benin, 650 kg.ha-1 compared to a potential yield of 4 kg.ha-1 for most cultivated varieties (Dansi *et al.*, 2012). The low yield may be attributed to various factors, such as competition among native Rhizobia in the soil, some of which have been found to be ineffective on Bambara groundnut (Basu *et al.*, 2007). Therefore, inoculation with compatible and appropriate Rhizobium strains is important where there is a small population of native compatible Rhizobium strains (Abd-Alla *et al.*, 2014). Studies carried out in different countries have shown the importance of legume crop inoculation by efficient Rhizobia strains. Indeed, inoculation with effective Bradyrhizobium strains improved productivity of soybean (*Glycine max*) (Zoundji *et al.*, 2015; Islam *et al.*, 2017; N'gbesso *et al.*, 2017; Houngnandan *et al.*, 2020); cowpea (Aboubacar *et al.*, 2013); groundnut (Sajid *et al.*, 2011; Didagbé *et al.*, 2014). Researchers (Musa *et al.*, 2016; Gomoung *et al.*, 2017; Ikenganyia *et al.,* 2017) reported positive response of Bambara groundnut to the inoculation with effective rhizobium strains.

Information on the crop in climatic conditions of Benin is limited (Gbaguidi *et al.*, 2018) and therefore evaluation of the effects of native rhizobium strains on Bambara groundnut varieties for yield and N fixation could provide information on its potentialities. More recently, Zoundji *et al.* (2022) screened some native rhizobium strains associated with Bambara groundnut in greenhouse conditions. Therefore, this study aimed to evaluate the effect of different strains of Rhizobium identified on the growth and productivity of three Bambara groundnut varieties in farmers' field conditions in semiarid regions of Benin. The outcomes of the study provide farmers with rational solutions to transform Bambara groundnut cultivation, foster sustainable agricultural practices, promote food security, and contribute to the overall well-being of farming communities in Benin and beyond.

Materials and methods

Study areas

This study was carried out in two Agro-Ecological Zones (AEZ) of Benin: *(i)* West Atacora Zone (AEZ 4) and *(ii)* Cotton Zone of central Benin (AEZ 5). The AEZ 4 is in northern Benin and characterized by a Sudanian climate with a unimodal rainfall pattern and an annual average rainfall ranging between 800 and 1500 mm (Hounsou, 2013). The dominant soil types in this AEZ include Leptosols, Aeronosols and Fluvisols. The AEZ 5 is in central Benin with a Sudano-Guinean climate, characterized by a bimodal rainfall pattern, with a tendency towards the Sudanian climate type in the northern part of the AEZ. The dominant soil types include Leptosols, Luvisols, Acrisols and Fluvisols.

In each zone, six farmer's fields were selected for the trials. The fields where the trial was carried out had maize as a previous crop. The experiments were conducted on Fluvisols in both AEZ. A baseline soil fertility reference was collected along the diagonal of the field at a depth of 0–20 cm and analysed at the laboratory of soil science of the University of Abomey-Calavi in Benin. The Kjeldahl nitrogen fixation method (Kjeldahl, 1883) was used to determine total nitrogen. Available phosphorus was estimated using the Bray I method (Bray and Kurtz, 1945). Exchangeable potassium was extracted using 1 mol/L neutral ammonium acetate and determined by the atomic absorption spectrometry method. Soil pH water (1:2.5) was determined following Mathieu and Pieltain's (2003) protocol. Particle size was determined using Robinson's method (Robinson, 1922). Soil total C was measured using the Walkley and Black oxidation method (Walkley and Black, 1934). Soils characteristics of the farmers' fields are reported in Table 1. The soil in both AEZ was slightly acid, poor in organic carbon, total nitrogen, and available phosphorus. In AEZ 4, the soil was sandy-clay-loam while the soil texture in AEZ 5 was loam-sand.

Experimental design

Two endogenous strains LMSEM312 and LMSEM338 and one reference strain (USDA110) were tested on three Bambara groundnut varieties i.e., Variety 1: *Azigokouiwéwékpè* (beige colour); Variety 2: Azigokouivovo (red colour); Variety 3: *Azigokouiwéwékpoguè* (beige colour spotted with red colour) and uninoculated plot was considered as control. In each AEZ, plots were arranged as a Randomized Complete Block design with six repetitions. The modalities of these factors (variety and inoculation) were combined to form twelve treatments (plots). Each plot was measured 10 m × 6 m. To prevent potential contamination between adjacent treatments, buffer zones of 2 m were installed around each plot. The space between each row was 0.75 m and the distance between plants was 0.15 m. Three seeds were initially sown in each hole and 14 days later, they were thinned to two. Prior to sowing, Bambara groundnut seeds were coated with the appropriate inoculum for each treatment. Weeding was done manually by hand to avoid contamination between treatments; and plots were maintained weed free throughout the experiment period. The plots did not receive any mineral or organic fertilizers.

Table 1. Physico-chemical properties of the soil before the starting of the experiment

Zones	pH (H_2O)	OM (%)	C (%)	N (%)	Available P (ppm)	Sand (%)	Silt (%)	Clay (%)
AEZ 4	6.2	0.93	0.54	0.03	13.57	65.59	11.08	23.33
AEZ 5	6.2	0.81	0.47	0.02	9.8	81.6	9.50	8.9

AEZ 4: West Atacora zone; AEZ 5: Cotton zone of central Benin; OM: Organic Matter; C: Carbon; N: Nitrogen; P: Phosphorus

Data collection

Plants heights and number of leaves were measured on the 15th, 22nd, 29th, 36th, and 43rd days after sowing (DAS) on 12 plants randomly selected on the central seed lines. Shoot and root biomass were collected at flowering stage (50 DAS). On each plot, the plants inside a density square of 2.25 m² were gently dug up and the roots were carefully separated from the above-ground biomass. For each plant all roots and 300 g above-ground biomass sample were collected, packaged, and labelled. In the laboratory, roots were washed, and nodules were removed and counted. The above-ground biomass root and nodules of the plants were oven-dried at 65°C for 72 h and dry matter was determined using the method inspired by Sharma *et al.* (2011). Nitrogen content was analysed using Kjeldahl method (Kjeldahl, 1883). At maturity, grains and straws were harvested on 3 x 3 m² area per plot and samples were collected dried and weighed. Total N in plant tissue samples were determined by Kjeldahl method. Biological Nitrogen Fixation was estimated from nitrogen accumulated using a polynomial regression equation (1), where Y represents biological nitrogen fixation and X the amount of nitrogen accumulated per hectare (Bado *et al.*, 2018).

$$Y = 0.0127X^2 - 0.5354X + 17.441 \quad (1)$$

Statistical analysis

The data were first checked for normality and homoscedasticity using Shapiro–Wilk test (Shapiro and Wilk 1965) and Bartlett's test (Bartlett 1937), respectively. Statistical analysis was conducted in SAS version 9.4 using the PROC MIXED procedure. Response variables such as number of leaves, height, shoot and root dry weight, nodulation, nitrogen uptake, amount of nitrogen derived from atmosphere and yield of Bambara groundnut were modelled against fixed effect variables of variety, inoculation, varieties, and their interaction within each AEZ. Replication was considered as a random effect. Means separation was performed using Student-Newman-Keuls (SNK) test with a significant threshold of 5%. As the study aimed at identifying optimal strains of rhizobium for Bambara groundnut production, only the interactive effect of Varieties*Inoculation was reported.

Results

Effect of rhizobial inoculation on growth parameters of bambara groundnut varieties

Number of leaves

The interactive effect of Varieties*Inoculation was significant ($p < 1$ %) on leaves number in AEZ 5 only at 29 days after sowing (DAS) and in both zones at 43 DAS (Table 2). At 29 DAS, all Bambara groundnut varieties showed positive response to inoculation with Rhizobium strains and the leaves number was greater under inoculated treatments compared to control. At 43 DAS, inoculation with the Rhizobium strain LMSEM338 increased leaves number by at least 35% compared to control for all the studied Bambara groundnut varieties In the AEZ 4, V2 and V3 inoculated with LMSEM338 yielded the highest number of leaves (54 leaves plant^{-1} and 53 leaves plant-1 respectively). In the AEZ 5, the high leave number was observed with the Rhizobium strain LMSEM 338 for V2 (52 leaves plant-1) and V3 (51 leaves plant^{-1}) while Rhizobium strain LMSEM312 yielded the higher leaves number with V1 (49 leaves plant^{-1}).

Plant height

Inoculation had significantly improved ($p < 1$ %) Bambara groundnut plants height at 29 DAS and 43DAS (Table 3). At 43 DAS, variety 2 and variety 3 had the highest heights (29 cm and 28 cm respectively) compared to variety 1 (24cm) In AEZ 4. Inoculation increased plant height with growth rates of 5.73%; 11% and 18% respectively for strains USDA110, LMSEM312 and LMSEM338. In AEZ 5, the height of Bambara groundnut ranged between 26 cm (V1) and 29 cm (V2). The combined effect of inoculation and variety was not significant on the height of different varieties.

Shoot and root dry weight at 50 DAS

Inoculation with different strains had a significant effect (p 1 ‰) on shoot and root dry weight of Bambara groundnut plants at 50 DAS (Table 4). Inoculation of each variety with LMSEM 338 strain showed the best results for varieties 2 and 3 in the two areas. The interactive effect of variety and inoculation also significantly ($p < 1$ %) affected the shoot and root dry weight for each variety. Variety 3 combined with LMSEM 338 improved shoot dry weight by 37% and 30% respectively in AEZ 4 and in AEZ 5. Shoot dry weight was improved by 27% and 26% respectively in AEZ 4 and in the AEZ 5 under the interaction of variety 2 and strain LMSEM338. However, strain LMSEM312 gave greater shoot and rood dry weight with variety 1 in AEZ 5 compared to other varieties. Similar trends were observed on root dry weight.

Effect of rhizobial inoculation nodulation of Bambara groundnut varieties at 50 DAS

Nodulation was significantly affected by inoculation. Nodules were observed on all varieties, but they showed more nodules when they were inoculated with Rhizobium strains (Table 5). In AEZ 4, inoculation allowed Bambara groundnut varieties to produce on average of 20 to 30 nodules per plant while the controls gave an average of 12, i.e., a growth rate ranging from 67% to 142%. The varieties 2 and 3 inoculated with the strain LMSEM338 showed the best nodulation.

Table 2. Effect of inoculation with different Rhizobium strains on the number of leaves of Bambara groundnut

Varieties	Inoculation	Number of leaves (number plant^{-1})					
		15 DAS		29DAS		43DAS	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	4.63a	4.6a	14.8c	14.67c	36.67d	36.16c
	LMSEM312	5a	5.63a	15.7b	16.95a	42.2c	48.91a
	LMSEM338	4.83a	4.72a	16.67a	16.64a	50.17a	48.68a
	USDA110	4.82a	4.63a	16.5a	15.96a	44b	43.91b
V2	Control	4.63a	4.53a	15.33b	14.45c	39.33d	38.62c
	LMSEM312	4.83a	4.59a	15.83b	16.03a	44.17c	43.23b
	LMSEM338	5a	4.78a	17.33a	16.15a	52.33a	51.99a
	USDA110	4.82a	4.64a	16.33a	16.01a	49.17b	48.97a
V3	Control	4.63a	4.43a	16.33a	15.36b	41c	39.97c
	LMSEM312	5a	4.65a	17a	16.25a	46.16b	45.87b
	LMSEM338	5a	4.85a	17.67a	16.89a	53.83a	51.26a
	USDA110	4.81a	4.68a	17.17a	16.67a	48b	46.94b
P-Values	Zone	0.16ns		0.08ns		0.21ns	
	Varieties	2.14ns		4.2**		10.24**	
	Inoculation	1.45ns		3.93**		7.10**	
	Zone*Varieties	2.78ns		5.4**		16.8*	
	Zone*Inoculation	3.8**		7.1*		12.1**	
	Varieties*Inoculation	0.32ns		1.2ns		8.1**	
	Zone*Varieties*Inoculation	3.12ns		1.6ns		10.5ns	

Means followed by the same letter are not significantly different at $p < 0.05$ according to Student – Newman-Keuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

Table 3. Effect of rhizobial inoculation on the height of Bambara groundnut varieties

Varieties	Inoculation	Plant height (cm)					
		15 DAS		29 DAS		43 DAS	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	15.12b	15.07c	19.94c	17.88c	21.72c	21.35b
	LMSEM312	16.74b	16.57b	20.81c	22.76a	24.76c	26.64a
	LMSEM338	16.01b	15.75c	20.59c	21.45a	25.83b	26.1a
	USDA110	15.69b	15.21c	20.85c	21.34a	23.52c	25.98a
V2	Control	18.64a	16.37b	25.06a	22.07b	27.08b	25.31b
	LMSEM312	19.07a	17.98a	25.09a	23.41a	29.28a	28.04a
	LMSEM338	19.17a	18.01a	25.65a	23.97a	31.41a	29.34a
	USDA110	19.16a	17.87a	25.23a	23.87a	29.96a	28.21a
V3	Control	18.55a	16.79b	23.75b	20.65b	25.48c	24.84b
	LMSEM312	18.70a	18.16a	24.60a	22.48a	28.40b	27.57a
	LMSEM338	18.87a	18.67a	25.12a	23.99a	30.50a	28.62a
	USDA110	18.72a	17.62a	24.14a	23.04a	27.05b	26.94a
P-Values	Zone	0.01*		0.67*		0.45*	
	Varieties	52.1**		94.2**		87.1**	
	Inoculation	0.87*		1.87*		21.5**	
	Zone*Varieties	7.28ns		51.2**		51.9*	
	Zone*Inoculation	1.7**		6.9*		14.8**	
	Varieties*Inoculation	1.3ns		5.4ns		2.5*	
	Zone*Varieties*Inoculation	4.2ns		1.9ns		13.01ns	

Means followed by the same letter are not significantly different at $p < 0.05$ according to Student – NewmanKeuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

For example, LMSEM338 strain induced the highest nodules number (30 nodules per plant) and nodule dry weight (0.34 g.plant^{-1}) on variety 3. Similar trends were observed in the AEZ 5, but best nodulation was found on variety 1 inoculated with strain LMSEM312. Strain LMSEM312 combined with variety 1 gave 28 nodules which weighted 0.31 g. However, significant difference was observed between two areas for the nodulation and best values were observed in AEZ 4.

Effect of rhizobial inoculation on shoot dry weight, nitrogen uptake and amount of nitrogen derived from atmosphere of Bambara groundnut varieties.

Table 4. Effect of rhizobial inoculation on the shoot and root dry weight of Bambara groundnut varieties at 50 DAS

Varieties	Inoculation	Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	3.01i	3.01c	1.75f	1,31c
	LMSEM312	3.75g	4.74a	2.58d	2.96b
	LMSEM338	4.81b	4.34a	3.06b	2.56a
	USDA110	4.27e	4.48a	2.61d	2.48b
V2	Control	3.50h	3.28c	2.11e	1.99c
	LMSEM312	4.20e	4.08b	2.80c	2.78a
	LMSEM338	4.82b	4.42a	3.34a	3.01a
	USDA110	4.43d	4.36a	2.99b	2.61b
V3	Control	3.53h	3.48c	2.00e	1.72c
	LMSEM312	4.02f	3.92c	2.92b	2.77a
	LMSEM338	5.01a	4.96a	3.32a	2.92a
	USDA110	4.65c	4.29a	2.89b	2.75a
P-Values	Zone	0.07ns		0.1ns	
	Varieties	77.4***		31.17***	
	Inoculation	758.9***		229.93***	
	Zone*Varieties	4.30*		12.1*	
	Zone*Inoculation	1.17**		8.9*	
	Varieties*Inoculation	10.24***		7.86**	
	Zone*Varieties*Inoculation	0.06ns		1.4ns	

Means followed by the same letter are not significantly different at p < 0.05 according to Student – NewmanKeuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

Table 5. Effect of rhizobial inoculation on nodulation of Bambara groundnut varieties at 50 DAS

Varieties	Inoculation	Nodule number (number.plant^{-1})		Nodule dry weight (g.plant^{-1})	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	11.00h	10.44d	0.17d	0.14c
	LMSEM312	19.83e	27.78a	0.28b	0.31a
	LMSEM338	29.67a	26.22b	0.32a	0.29a
	USDA110	22.33d	20.55c	0.28b	0.24b
V2	Control	12.33g	11.11d	0.17d	0.14c
	LMSEM312	20.83e	19.45c	0.24c	0.23b
	LMSEM338	29.77a	26.17a	0.33b	0.28a
	USDA110	24.50c	23.59b	0.28b	0.26a
V3	Control	12.83g	11.01d	0.16d	0.14c
	LMSEM312	18.50f	17.33c	0.28b	0.23b
	LMSEM338	29.90b	27.22a	0.34a	0.27a
	USDA110	25.00c	24.98b	0.28b	0.24b
P-Values	Zone	0.95*		0.1*	
	Varieties	45.55***		72.53***	
	Inoculation	236.83***		710.5***	
	Zone*Varieties	11.7*		7.11*	
	Zone*Inoculation	9.7**		1.9**	
	Varieties*Inoculation	3.91**		6.5**	
	Zone*Varieties*Inoculation	7.8ns		1.3ns	

Means followed by the same letter are not significantly different at p < 0.05 according to Student – NewmanKeuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

Inoculation significantly affected nitrogen uptake and amount of nitrogen derived from atmosphere of different Bambara groundnut varieties (Table 6). The maximum nitrogen uptake and amount of nitrogen derived from atmosphere was recorded with the strain LMSEM338. The shoot dry weight increased by 37%, 46% and 55% respectively for treatments with LMSEM312, USDA110 and LMSEM338 strains within each variety. The interaction between Rhizobium inoculation and varieties showed that greater shoot dry weight, nitrogen uptake and amount of nitrogen derived from atmosphere was recorded with rhizobium inoculated plots in variety 3 and minimum values were obtained on control treatment and the best values were found in the AEZ 4.

Effect of rhizobial inoculation on pod and grain yield of Bambara groundnut varieties

Inoculation showed a significant effect ($p < 0.001$ à $p < 0.01$) on pod and grain yield of the different varieties (Table 7).

Table 6. Effect of rhizobial inoculation on shoot dry matter, nitrogen uptake and amount of nitrogen derived from atmosphere (NDFA) of Bambara groundnut varieties

Varieties	Inoculation	Shoot dry weight (kg ha^{-1})		Nitrogen uptake (kg ha^{-1})		Amount of NDFA (kg ha^{-1})	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	800.76f	800.35e	19.11g	18.25f	11.98e	10.89f
	LMSEM312	1391.50d	1790.61a	40.30d	51.72b	16.65d	27.95b
	LMSEM338	1835.28b	1779.24a	61.05a	60.72a	32.13a	31.56a
	USDA110	1415.80d	1403.51b	43.41c	43.25c	18.26c	18.07d
V2	Control	1082.51e	1035.45d	27.84f	26.75e	12.45e	12.29e
	LMSEM312	1348.39d	1320.19c	38.49e	33.83d	15.87d	14.35e
	LMSEM338	1888.61a	1794.28a	63.43a	62.25a	34.70a	32.51a
	USDA110	1509.60c	1490.60b	46.45c	48.83b	20.02c	30.37a
V3	Control	1084.46e	1075.87d	28.62f	27.59d	12.54e	12.4e
	LMSEM312	1350.65d	1294.33c	41.09d	39.58c	16.96d	16.87d
	LMSEM338	1894.68b	1782.94a	62.44a	60.15a	33.70a	31.96a
	USDA110	1562.98c	1523.87b	50.07b	48.97b	22.54b	21.47c
P-Values	Zone	2.42*		2.84*		1.98*	
	Varieties	134.11***		91.14***		4.54*	
	Inoculation	932.09*		436.38***		138.37***	
	Zone*Varieties	62.1*		47.7**		37.57**	
	Zone*Inoculation	9.2*		12.3**		10.41*	
	Varieties*Inoculation	4.08**		23.74**		2.32*	
	Zone*Varieties*Inoculation	2.4ns		5.6ns		4.9ns	

Means followed by the same letter are not significantly different at $p < 0.05$ according to Student – NewmanKeuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

Table 7. Effect of rhizobial inoculation on pod and grain yield of Bambara groundnut varieties

Varieties	Inoculation	Pod yield (kg.ha^{-1})		Grain yield (kg.ha^{-1})	
		AEZ 4	AEZ 5	AEZ 4	AEZ 5
V1	Control	760.67g	722.0e	616.63g	518.13e
	LMSEM312	1241.81e	1788.35a	1047.94e	1596.89a
	LMSEM338	1782.77c	1766.73a	1557.76c	1364.83b
	USDA110	1456.16d	1368.45c	1244.80d	1372.24b
V2	Control	911.83f	910.72d	703.46f	659.44e
	LMSEM312	1429.87d	1419.7c	1198.63d	1113.57c
	LMSEM338	2024.60a	1741.7a	1819.63b	1489.29a
	USDA110	1786.71c	1691.7b	1570.44c	953.2d
V3	Control	941.29f	890.8d	762.25f	735.46d
	LMSEM312	1468.13d	1330.9c	1229.59d	1206.85c
	LMSEM338	2164.67a	1786.09a	1918.54a	1558.94a
	USDA110	1995.98b	1733.3a	1796.01b	1557.25a

P-Values	Zone	1.02*	2.3*
	Varieties	264.63***	194.68***
	Inoculation	1630.24***	1289.9***
	Zone*Varieties	16.8*	18.9*
	Zone*Inoculation	12.34*	14.1*
	Varieties*Inoculation	15.31***	17.52***
	Zone*Varieties*Inoculation	23.4ns	19.7ns

Means followed by the same letter are not significantly different at p < 0.05 according to Student – NewmanKeuls test. ns: not significant (p>5 %); *: significant (p < 5 %) **: Significant (p < 1 %) ; *** : Significant (p < 1 ‰). V1: Variety 1 (Azigokouiwéwékpè); V2: Variety 2 (Azigokouivovo); V3: Variety 3 (Azigokouiwéwékpoguè)

For pod yield, an increase of 508.68 kg ha-1, 875.02 kg ha-1 and 1119.42 kg ha-1 was noted on treatments with strains LMSEM312, USDA110 and LMSEM338 respectively. The interactive effect between varieties and inoculation was also significant. Variety 3 inoculated with the strain LMSEM338 showed the highest yield compared to other treatments. Same trend was found on grain yield. In AEZ 4, pod yield and grain yield ranged between 760.67 kg ha^{-1} 616.63 kg ha^{-1} (Variety 1 uninoculated) and 2164.67 and 1918.64 kg ha^{-1} (variety 3 inoculated with strain LMSEM 338) respectively. In AEZ 5, pod yield varied from 722 kg ha-1 (variety 1 uninoculated) to 1788.35 kg ha^{-1} (variety inoculated with strain LMSEM312) when grain yield ranged between 518.13 kg ha^{-1} and 1596.89 kg ha^{-1} for variety 1 uninoculated and variety inoculated with strain LMSEM312 respectively.

Discussion

The study aimed to identify the efficient rhizobium strain for enhancing the agronomic performance and productivity of three Bambara groundnut varieties, contributing to food security and poverty reduction in Benin. Inoculation with rhizobium strains positively affected Bambara groundnut growth parameters. The use of effective Rhizobia strains in leguminous crops has been associated with the production of plant-growth-promoting hormones such as 3-indoleacetic acid (IAA), contributing to enhanced plant growth and development (Jaiswal *et al.*, 2021). These results align with studies by Gomoung *et al.* (2017) and Ikenganyia *et al.* (2017) that illustrated the positive effects of rhizobia strains on Bambara groundnut growth parameters. Similarly, Didagbé *et al.* (2014) and Zoundji *et al.* (2015) reported significant influences on aboveground and root biomass production for groundnut and soybean in Benin. Aboveground biomass is correlated with large root system development, better rhizosphere exploration and improved nutrient uptake (Elhaissoufi *et al.*, 2020).

Inoculation significantly influenced shoot and root dry weight, nodule number, and nodule dry weight, with strain LMSEM338 showing favourable results for varieties 2 (Azigokouivovo) and 3 (Azigokouiwéwékpoguè). Growth rates varied with different Rhizobium strains, suggesting strain-specific influences on Bambara groundnut plant growth parameters. Strain LMSEM338 in AEZ 4 and LMSEM 312 in AEZ 5 exhibited higher plant growth rates, emphasizing the need to consider environmental factors in assessing inoculation efficiency. The variations between agro-ecological zones suggested that environmental conditions play a prominent role in inoculation effectiveness on biomass production. The strain LMSEM338 consistently produced the highest values in nitrogen uptake and atmospheric nitrogen contribution, with variety 3 showing the greatest responses to Rhizobium inoculation. The interactive effects between zones, varieties, and inoculation highlight the complexity of interactions influencing nitrogen dynamics in Bambara groundnut production.

The presence of nodule in non-inoculated plot (control), thought in low quantity is attributable to natural presence of soil microorganisms in the soil. Even in the deliberate absence of Rhizobium inoculation, soils often harbour diverse populations of naturally occurring rhizobia (Sánchez-Navarro *et al.*, 2020).

These indigenous soil microorganisms may establish symbiotic relationships with leguminous plants, leading to the formation of nodules. However, these rhizobia may be in low population density, not be as efficient or not specialized as the ones applied through inoculation, leading to low nodulation and less biological nitrogen fixation (N'Gbesso *et al.*, 2017).

The study acknowledges that nodules alone may not accurately reflect the nitrogen-fixing abilities of a rhizobial strain. Various rhizobia strains can form numerous nodules without efficiently fixing nitrogen, emphasizing the importance of assessing functional effectiveness for improving nitrogen fixation in legumes like Bambara groundnut (Athul *et al.*, 2022). The large amplitudes of dry nodule production obtained with variety 3 inoculated with the LMSEM338 strain in northern Benin and variety 1 inoculated with LMSEM 312 in the Centre, indicate that there is high compatible between the varieties and strains and illustrates the interactive effects between zones, varieties, and rhizobium strain.

Native rhizobia strains exhibited superior performance in root and shoot dry weight compared to introduced strains and controls, consistent with previous studies. The superior adaptability of native isolates to the local agroecological environment underscores their potential as effective rhizobia inoculants for enhancing Bambara groundnut production (Asante *et al.*, 2020; Nyaga and Njeru, 2020). The results suggest that reintroducing well-adapted rhizobia strains enhances nodule formation aligning with the concept that adaptability to the local environment is crucial for successful rhizobia-legume symbiosis (Matse *et al.*, 2020).

Inoculation improved yield components for all varieties compared to the control, significantly influencing biomass yield and nitrogen accumulation in aboveground biomass and seeds. Biomass production is vital for nutrient recovery and soil organic matter replenishment when the crop residues are incorporated to the soil. Regarding pod yield, all three strains significantly influenced yield compared to controls. Differences in pod yields with different strains and different varieties underscored the strain-specific responses of Bambara groundnut varieties to inoculation (N'gbesso *et al.*, 2017; Njeru *et al.*, 2020). The study implies that exploiting these varietal responses could be pivotal for developing Bambara groundnut varieties with high nitrogen fixation abilities adapted to each agroecological zone, emphasizing the importance of tailored inoculation strategies based on Bambara groundnut genotypes.

Conclusion

This paper showed that inoculation with rhizobium strains significantly improved Bambara groundnut agronomic performance and yield parameters in central Benin. The observed significant effects of Rhizobia inoculation on various growth parameters and yield attributes underscore the potential of tailored inoculation strategies for specific cultivars and agroecological zones. Outstandingly, in AEZ 3, variety 3 (Azigokouiwéwékpoguè) exhibited the highest yield when inoculated with strain LMSEM 338, followed by varieties 2 (Azigikouivovo) and variety 1 (Azigokouiwéwékpè). In AEZ 5, Variety 1, inoculated with LMSEM 312, demonstrated superior yields, comparable to variety 3 (Azigokouiwéwékpoguè) inoculated with LMSEM 338. These findings suggest the potential use of specific strains in biofertilizer production, offering a sustainable approach to enhance Bambara groundnut yield in marginal soils with minimal inputs. Regional preferences should be considered, as Variety 2 (Azigikouivovo) and 3 (Azigokouiwéwékpoguè) prove more suitable for the North region (AEZ 4), while Variety 1 (Azigokouiwéwékpè) exhibits optimal performance in the Centre area (AEZ 5). However, caution is warranted in generalizing findings, as the study's pioneering exploration of indigenous rhizobia strains for Bambara groundnut inoculation in Benin may limit the conclusions to specific geographic areas, soil types, and climatic conditions. To strengthen the broader applicability of these findings, it is recommended to conduct further multiyear and multi-location research.

This will contribute to a comprehensive understanding of the potential benefits of rhizobia inoculation, ultimately supporting sustainable agricultural practices in smallholder semi-arid farming systems across West Africa.

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