Int.J.Curr.Microbiol.App.Sci (2026) *15*(1): 1-17

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 1 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1501.001

Characterization of Bacterial Rhizobial Communities Associated to three Provenances of Pigeon Pea (*Cajanus cajan* L.) Cultivated in Soils from three Different Regions in Senegal

Fatou NDOYE[1,3*], Tatiana Krasova-WADE[3], Aboubacry KANE[2,3], Diariatou NIANG[3], Seynabou NGOM[2,3], Papa Talybe Gueye SANE[2], Christian Valentin NADIEDINE[3], Mariama NGOM[2,3], Cheikh NDIAYE[2,3], Diegane DIOUF[1,3], Mame Oureye SY[2,3] and Abdala Gamby DIEDHIOU[2,3]

[1]*University of Sine Saloum El Hadj Ibrahima NIASS, Post Box 55, Kaolack, Senegal*
[2]*University of Cheikh Anta Diop, Post Box 5005, CP C10700, Dakar-Fann, Senegal*
[3]*Institute of Research for Development (IRD), UMR Eco&Sol, Joint Laboratory of Soil Microbiology, (LCM, IRD/UCAD/ISRA), Post Box 1386, Dakar, Senegal*

**Corresponding author*

Keywords

Neglected and Underutilized Species, Pigeon pea, Rhizobia, Diversity, Infectivity

Article Info

Received:
05 November 2025
Accepted:
22 December 2025
Available Online:
10 January 2026

A B S T R A C T

Pigeon pea (*Cajanus cajan* L.) is a legume with an important source of proteins, grown in several tropical and subtropical regions. However, despite its great importance, especially in tropical regions facing food and nutritional insecurity, pigeon pea remains one of the oldest and least valued food crops in West Africa, particularly in Senegal. The aim of this study was to contribute to a better understanding of the diversity of rhizobia associated with three pigeon pea provenances in three regions of Senegal (Fatick, Nioro and Kaffrine), with a view to their exploitation as microbial biofertilizers. Soil samples were collected from rhizosphere of pigeon pea plants in the three regions. Shadehouse trials were carried out to determine the most probable number of bacteria (MPN), as well as the phenotypic and genetic diversity of rhizobia by trapping with pigeon pea seeds from the three provenances. The results revealed a variability in MPN between the three study sites. Bacterial isolation from root nodules determined that pigeon pea associates more with fast-growing bacteria. A collection of 87 isolates was obtained from the plant nodules cultivated in the three sites. Amplification of the rDNA ITS region, followed by enzymatic digestion, disclosed 35 genetic profiles, including 4 common to Fatick and Kaffrine, 2 to Fatick and Kaffrine, 1 to Nioro and Kaffrine, 8 specific to Fatick, 8 to Kaffrine and 5 to Nioro. At the end of the infectivity test with 56 bacterial isolates, considered under controlled conditions, only 29 re-infected the host-plant. A positive effect of inoculation with some bacterial isolates (F12p, N23, N22, N5, K3 and K16p) was noted for chlorophyll content and certain plant growth parameters (nodule number and weight, shoot and root dry weight). This study underlined that some of the bacterial strains may be potential candidates for improving growth and productivity of pigeon pea in Senegal.

Introduction

From 1961 to nowadays, food supply per capita has increase by 30%; accompanied by a considerable rise in the use of mineral fertilizers. Currently, it is estimated that 733 million people are undernourished worldwide (FAO *et al*., 2020); and that approximately two billion people suffer from micronutrient deficiencies that make them more vulnerable to diseases, which can be a significant obstacle to economic growth (Ferreira *et al*., 2017). Africa remains one of the continents with the highest prevalence of undernourishment, affecting almost 21% of its population (Sukati, 2020). This phenomenon remains one of the major challenges facing the world today, as global population growth is projected to reach approximately 10.3 billion people by 2084 (Lam, 2025). However, about more than 50,000 edible worldwide wild plants considered, only 200 plant species are used to meet 90% of global food needs, along with a few staple crops such as wheat, rice, maize and millet (UNSCN, 2020). As one of the alternatives for nutritious and healthy foods, edible wild plants are of vital importance in meeting global food needs, particularly in Africa (Duguma, 2020). Therefore, harnessing the potential of many neglected or underutilized plant species could contribute to reaching the demand for increased food production and its diversification. This later might ensure the resilience of food system while improving environmental capacity (Baldermann *et al*., 2016). Promoting these plant species is thus one of the levers that can be used to address the challenge of food and nutritional security, as well as job creation and poverty alleviation (Falola *et al*., 2022). Pigeon pea (*Cajanus cajan,* L.) is one of these underutilized species, with huge potentials, which however, yet still untapped in West African countries such as Senegal. This plant species is a grain legume belonging to the *Fabaceae* family, cultivated in the tropical zones, including semi-arid ones. It is the sixth most important grain legume in the world (Pazhamala *et al*., 2015), with an estimated average annual production of 3.1 million tons, representing approximately 5% of global grain legume production. This classifies it fifth among grain legumes and contributes 33% of the nitrogen requirements for human nutrition (Fossou *et al*., 2012). Its protein content varies between 21% and 25%, and is sufficient to reach nutritional needs when mixed with other legumes and cereals (Jeevarathinam and Chelladurai, 2020). Pigeon pea is a legume closely linked to soil microorganisms, particularly rhizobium bacteria, with which it establishes a symbiotic relationship that allows it to fix significant amounts of atmospheric nitrogen (Khuntia *et al*., 2022). Furthermore, it has been demonstrated that harnessing the potential of biological nitrogen fixation and applying inoculum could considerably reduce the use of chemical nitrogen fertilizers in agricultural production systems (Diedhiou *et al*., 2022). The high nutritional value of pigeon pea, its exceptional capacity for soil fixation and regeneration, and its diverse uses for human and livestock, make it one of the most important crops in Africa. However, despite its great importance, especially in tropical regions facing food and nutritional insecurity, pigeon pea remains one of the oldest but undervalued food crops (Namuyiga *et al*., 2022). The proportion of pigeon pea produced in West Africa remains the lowest (around 600-700 kg/ha), despite a potential of 1200-300 kg/ha (Fatokimi and Tanimonure, 2021). Moreover, in Senegal, its genetic diversity, production techniques and uses remain largely unknown. As a result, it is relatively untapped and undervalued. The overall objective of this study is to contribute to a better understanding of the diversity of rhizobia associated to three pigeon pea provenances in three different regions of Senegal with a view to their use as microbial biofertilizers. Specifically, the study will determine the density and diversity of the rhizobia population compatible with three pigeon pea provenances cultivated in soils from three regions (Kaffrine, Fatick, and Nioro). In the other hand, the effectiveness of selected isolates on pigeon pea plants growth regardless of soil origin and seed provenance will be evaluated. The previous hypothesis was that site characteristics and seed provenance might influence bacterial rhizobia density and diversity associated to the pigeon pea.

Materials and Methods

Study site and sampling

The study was conducted with soils from three regions of the Groundnut Basin of Senegal: Fatick, Nioro and Kaffrine (Plate 1). In these three regions, soil samples were collected from the Kaydara farm (in Fatick), from a home garden (in Nioro) and from the Natangué farm (in Kaffrine). The Kaydara farm is located in the municipality of Fimela (14.2° N latitude and 16.3° W longitude), in the Sine Saloum Delta (Fatick). Temperatures generally range from 20°C to 35°C. Annual rainfall is around 487 mm to 667 mm. Nioro is a locality in Senegal situated in the Kaolack region (13.5667° N latitude and 10.1333° W longitude). Annual temperatures range from 18°C to 40°C, with an average

annual rainfall around 864 mm. The Natangué farm is located in Sagna, situated in the Malem Hodar Department in the Kaffrine region (14°05'12.5"N 15°23'00.0"W). The temperatures range from 26°C to 39°C during the day. There are 11 hours of sunshine per day. Annual rainfall varies between 765 mm and 846 mm.

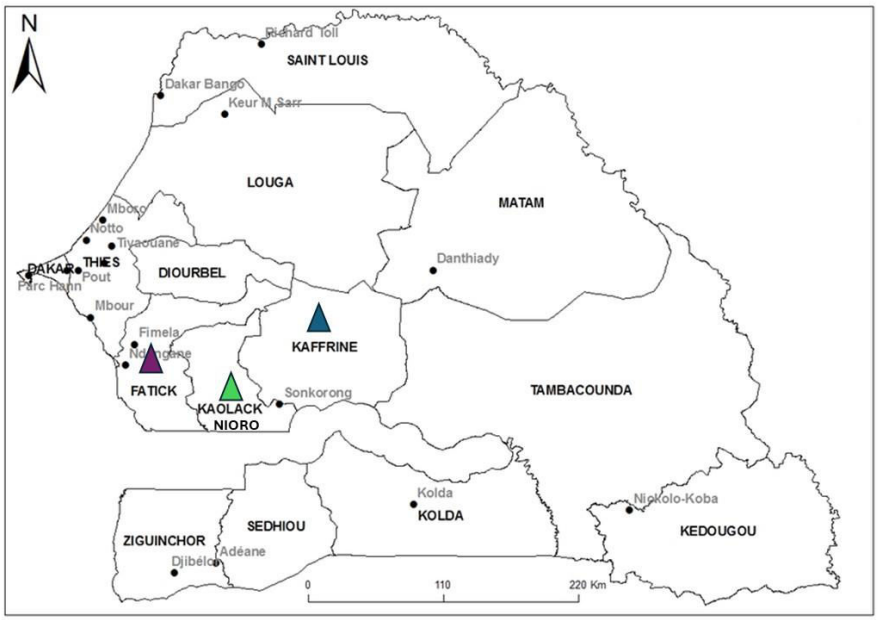

Plate.1 Study sites in Senegal (Sane *et al*., 2016 adapted)

In each of the three pigeon pea plantations located in the three targeted regions, 10 plants were randomly selected. Around each plant, three sampling points were chosen, and soil samples were collected from each point at a depth of 0–20 cm. The three soil samples from each plant were mixed to create a composite soil sample. The soil samples were then placed in plastic bags and taken to the Joint Laboratory of Soil Microbiology IRD-ISRA-UCAD (Bel Air, Dakar) for microbiological analysis.

In each study site, about 100 g of seeds of pigeon pea were collected from plants and kept at 4°C at the laboratory.

Soil physicochemical characterization

The composite soil samples were analyzed at the Soil-Plant and Water Testing Laboratory of CNRA–ISRA (Bambey, Senegal). The particle size analysis included the percentages of coarse sand, medium sand, fine sand, clay, and silt, and were determined with standard protocols. The chemical characteristics related to the contents of essential mineral elements, namely total phosphorus, total nitrogen, total carbon and exchangeable bases (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) were determined as described in Faye *et al*. (2021). pH (H_2O) was measured using the protocol of Mathieu and Pieltain (2003).

Estimation of the Most Probable Number of nodule bacteria

The density of natural rhizobium populations in the soils from the three regions was estimated using the Most Probable Number (MPN) method, based on the infection of host plant by their symbionts (Vincent, 1970). Pre-germinated pigeon pea seeds were transplanted into pots containing sterile soil from Sangalkam and then inoculated with successive dilutions (from 10^{-1} to 10^{-6}) of collected soil suspensions. For each dilution, two plants were inoculated, and four replicates were performed. Four uninoculated plant controls were also included. After 45 days of growth in a shadehouse conditions, the presence or absence of nodules was recorded.

The number of bacteria was calculated using the statistical table of Fisher and Yates (1963), according to the following formula: MPN = m x v / d with m: most probable number based on infection results; v: volume inoculated per plant (10 mL) and d: lowest dilution (10^{-1})

in this study. This approach allows a quantitative estimation of the population of symbiotically active rhizobia in the soils.

Trapping culture for rhizobial strains isolation in soils

Rhizobium trapping was carried out according to the methods of Vincent (1970) and Somasegaran and Hoben (1994). The experiment was conducted in a shadehouse in pots filled with 1 kg of soil from each study site. For each soil, pre-germinated pigeon pea seeds from the three provenances were transplanted with three replicates per provenance, for a total of nine pots per soil site. After 3 months of cultivation, plants were harvested and root nodules were collected in Eppendorf tubes for bacterial isolation and characterization.

Isolation and phenotypic characterization of rhizobial strains

Isolation and purification of nodules: Under a laminar flow hood, the nodules of each plant, washed with sterile distilled water, were disinfected by soaking in 70% alcohol for 3 minutes and then in 3% Calcium Hypochlorite for 1 minute. Then, they were rinsed 6 times with sterile distilled water. The nodules were then placed in sterile Eppendorf tubes and crushed using a sterile flask. The bacteria were isolated by pipetting a small amount of homogenate solution from each tube. The crushed nodule suspensions were inoculated onto YMA (Yeast-Mannitol-Agar) medium (Vincent, 1970) in Petri dishes using a platinum loop ignited with a Bunsen burner. Inoculation was performed using the four-quadrant method to obtain isolated colonies. The inoculated Petri dished were then incubated in an oven at 30 ± 1°C for 5 days. Subsequently, the isolated colonies were successively inoculated into Petri dishes at varying time intervals for their purification.

Phenotypic characterization of nodule bacteria: After purification of the bacterial colonies, several phenotypic characteristics were determined such as the diameter, shape, surface area, height, consistency, color, outline and growth at 36°C of the colonies.

Molecular characterization of rhizobial strains: DNA extraction was made on the purified bacteria suspension by heat shock. Amplification of the 16S–23S intergenic region of the rDNA was performed by PCR using primers FGPS1490-72 and FGPL-38 (Normand *et al*., 1996). Amplification was performed using a Gene AMP PCR System 2400 thermocycler (Perkin Elmer) according to the modify program described by Krasova-Wade *et al*. (2001) with initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, primer hybridization at 55°C for 30 s, and elongation at 72°C for 1 min. A final elongation step at 72°C for 7 min was applied to ensure complete synthesis of the fragments. Finally, the temperature was maintained at 4°C for the preservation of the PCR products. For each amplification reaction, a microtube containing only the reaction medium without DNA was used as a negative control. Confirmation of the PCR products was performed by 1% agarose gel electrophoresis.

Restriction fragment length polymorphism (RFLP) analysis was then conducted using *Hae*III and *Msp*I enzymes, with migration on 2.5% agarose gel. This approach allowed for the differentiation of the molecular profiles of the rhizobial isolates.

Infectivity test of nodule bacterial isolates

Nodulation tests were performed according to the protocol of Gibson (1963) in culture tubes containing sterile Jensen's medium. Pre-germinated pigeon pea seedlings were transplanted into 22 x 220 mm Gibson tubes containing 30 mL of sterile, sloping Jensen nutrient medium and sterile distilled water, with one seedling per tube. A capped opening was left in the tube to facilitate watering. The racks containing the Gibson tubes were wrapped in aluminum foil, and the entire setup was placed in a humid, confined atmosphere for 48 hours to ensure spontaneous seed coat release.

The isolated bacteria were cultured in liquid YM (Yeast Malt) medium for 72 hours, and 1 mL of each bacterial culture was used to inoculate the roots of 48 hours previously transplanted seedlings. Each treatment was repeated three times, and three uninoculated plants per site served as controls. For each pigeon pea provenance, bacterial isolates from the same site were used for inoculation. All tubes containing plants were subjected to intermittent lightening (16 hours of light and 8 hours of darkness) for 45 days in a growth chamber at a temperature of 28 ± 1°C. At the end of the experiment, the number of nodules, plant shoot and root dry biomass as well as chlorophyll content (measured with a SPAD-502 Plus) were evaluated to determine the symbiotic effectiveness of the bacterial isolates.

Statistical analysis

Data were subjected to one-way or two-way analysis of variance (ANOVA), and means were compared using Fisher's LSD test at the 5% threshold ($p \leq 0.05$), with XLSTAT software (version 2016). Principal Component Analysis (PCA) method was performed to detect the correlations between treatments in each soil site using Pithon 3.12 software (https://www.python.org/downloads/release/python-3120/).

Results and Discussion

Soil physicochemical characteristics

The results of the physicochemical analysis of soils are recorded in Table 1. The quantities of the different particles (clay, silt and sand) found in the soils allowed to deduce that all three soils are sandy. The soils of Fatick and Kaffrine are more acidic (pH=6.19 and 6.12) than that of Nioro (pH=7.67). The carbon content is higher in the soil of Fatick (0.59%) and lower in that of Kaffrine (0.14%). Regarding nitrogen, the highest level was observed in the soil of Nioro (0.09%) and the lowest in that of Kaffrine (0.04%). The amount of total phosphorus was greater in soil from Nioro (23.84 ppm) and the lowest in that of Fatick (0.12 ppm), Exchangeable bases (Ca^{2+}, Mg^{2+} and Na^{+}) are also higher in the soil of Nioro.

Most Probable Number of rhizobia and bacterial density in plant nodules

The most probable number of rhizobia (Table 2) showed significant variations among the three study sites. Estimated bacterial densities were 7×10^6 bacteria/g of dry soil in Kaffrine, 6.7×10^6 in Fatick, and only 3.1×10^4 in Nioro. These results indicate strong heterogeneity in the rhizobium population capable of nodulating pigeon peas across the different sites.

Results also revealed that the total number of bacteria infected root nodules of pigeon pea plant varied among study sites. The latter was higher in root nodules from Fatick and Kaffrine compated to that from Nioro (Table 2).

Phenotypic diversity of bacterial isolates

On YMA medium, bacterial isolates fall into two groups based on their growth rate: 91% of isolates are fast-growing bacteria (colonies visible in less than 3 days) and 9% are slow-growing bacteria (colonies visible between 3 to 5 days). This predominance is observed for all three sites. It is consistent with the higher competitive capacity of these strains, particularly in pigeon pea, which appears to naturally favor fast-growing bacteria during its vegetative phase.

The morphological characteristics of the bacterial colonies, including color, edge, relief and viscosity, revealed diversity among the isolates, with no single morphological type being specific to any one site (Figure 1). All the isolated bacteria were circular in shape, 91% showed rapid growth at 36°C, 77% had a creamy consistency, and 63% had a regular outline. However, the other parameters varied between groups. This phenotypic variability reflects a biological diversity potentially linked to the local rhizosphere environment and site-specific microbial interactions.

Genetic diversity of bacterial isolates

Digestion of the 87 amplicons from the three sites (37 for Fatick, 17 for Nioro and 33 for Kaffrine) using the restriction enzyme *Msp*I generated 14 profiles or groups for Fatick, 8 for Nioro and 12 for Kaffrine. Among them, 8 profiles or groups were specific to Fatick, 5 to Nioro and 8 to Kaffrine (Figure 2A). It was observed that some groups with the same morphological traits had the same RFLP profile, while others had not been cleaved by the enzyme. The RFLP profiles obtained with the *Msp*I enzyme were confirmed by the restriction enzyme *Hae*III. The soils of Fatick and Kaffrine shared 4 genetic profiles, Fatick and Nioro shared 2, and Nioro and Kaffrine shared 1 genetic group (Figure 2B).

Infectivity and symbiotic efficiency of rhizobial isolates

At the end of the experiment, the examination of plant root systems revealed that, of the 53 isolates tested, only 28 reinfected the host-plant, representing a rate of 52.83% (Table 3, Plate 2). The uninoculated plants did not develop nodules. The nodules are located on the lateral roots of the plants. Specifically, in Fatick, 6 of the 21 isolates tested reinfected the plants, while in Nioro, 10 of the 13 isolates reinfected the plants. Finally, in Kaffrine, 12 of the 19 tested isolates are positive.

Table.1 Soil physicochemical characteristics of study sites

Components	Study sites		
	Fatick	Nioro	Kaffrine
Clay (%)	3.13	3.88	3.94
Silt (%)	1.91	4.26	5.27
Sand (%)	94.96	82.87	9079
Total Carbon (%)	0.59	0.33	0.14
Total Nitrogen (%)	0.08	0.09	0.04
Total Phosphorus (ppm)	0.12	23.84	0.22
K^+ (Cmol/kg)	0.013	0.009	0.013
Na^+ (Cmol/kg)	0.047	0.054	0.049
Mg^{2+}(Cmol/kg)	1.400	4.800	0.20
Ca^{2+}(Cmol/kg)	2.000	6.000	1.200
pH (H_2O)	6.19	7.67	6.12

Table.2 Density of bacteria in soils and root nodules

Soil sites	Most Probable Number (MPN) (Number of bacteria /g of soil)	Total number of bacteria isolates in nodules
Fatick	**$6.7\ 10^6$**	**37**
Nioro	$3.1\ 10^4$	17
Kaffrine	**$7\ 10^6$**	**33**

Figure.1 Phenotypic properties of nodule bacterial isolates

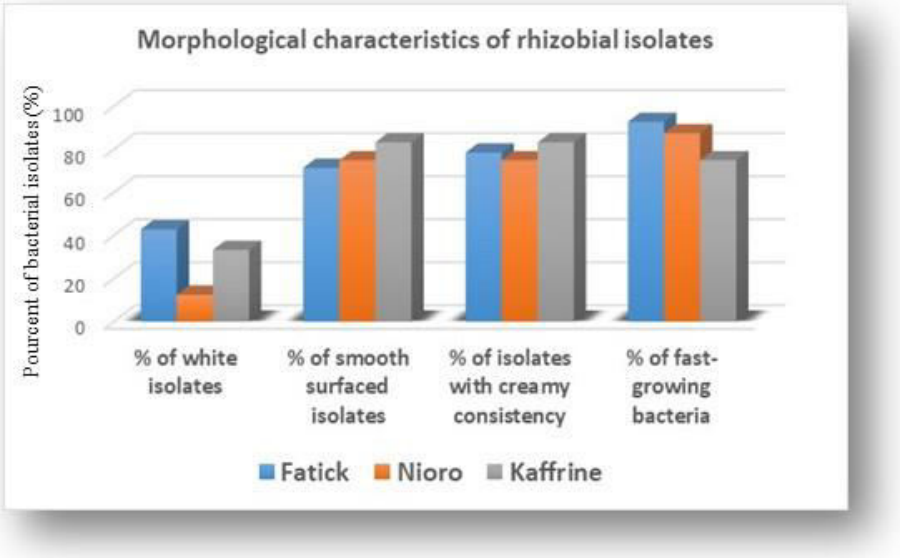

Figure.2 Number of 16S-23S rDNA RFLP groups on nodule bacterial isolates from the three geographical sites

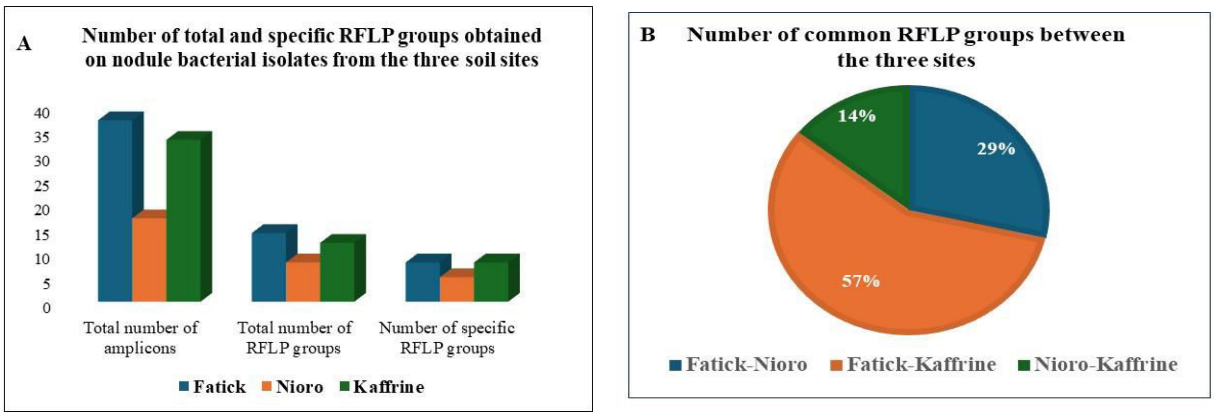

Figure.3 Principal Component Analysis on growth and symbiotic variables in Fatick soil

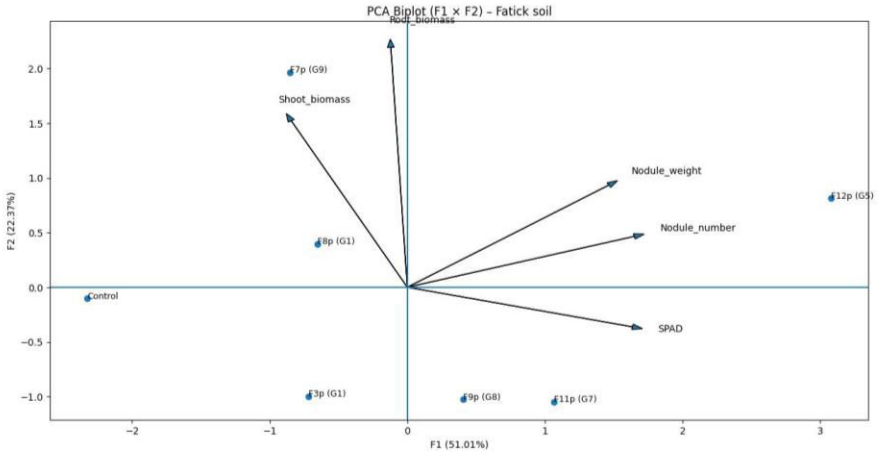

Figure.4 Principal Component Analysis on growth and symbiotic variables in Nioro soil

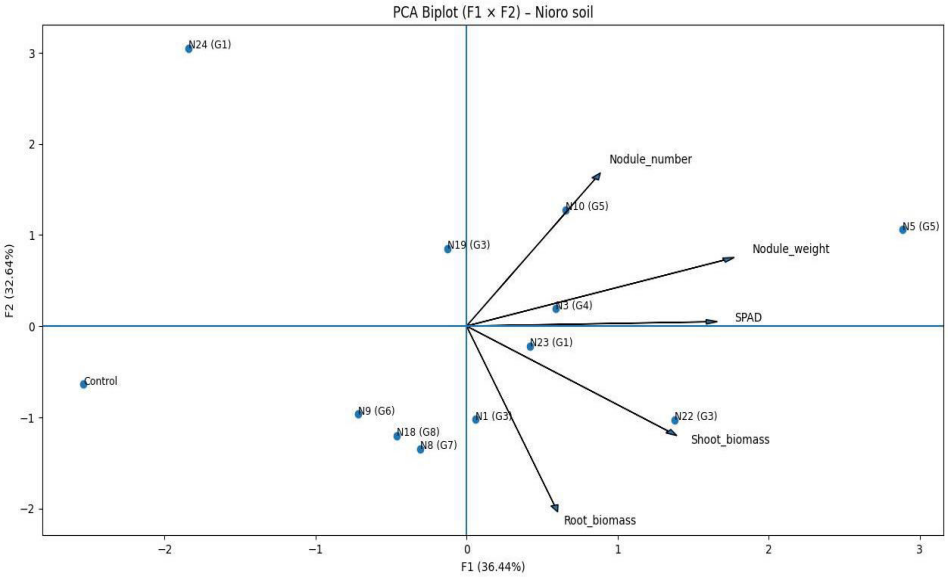

Figure.5 Principal Component Analysis on growth and symbiotic variables in Kaffrine soil

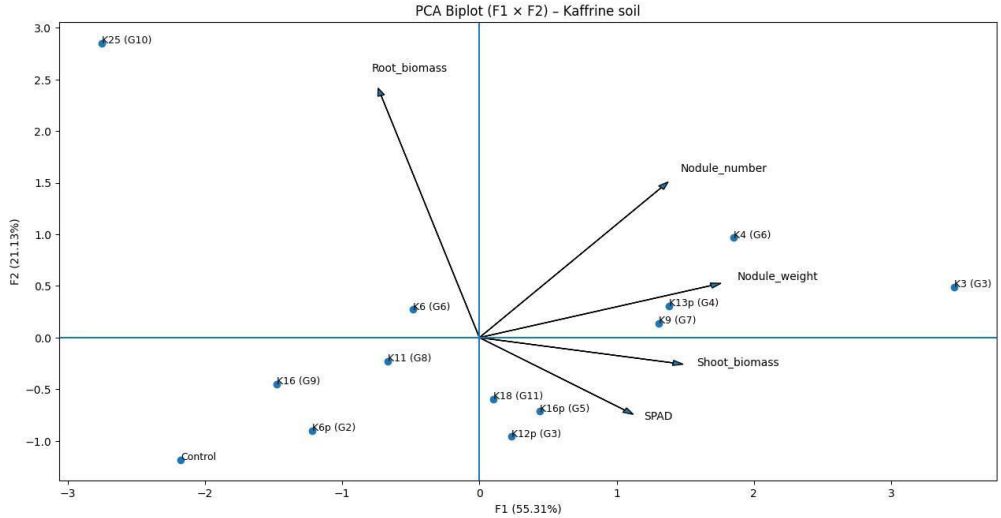

Plate.2 Morphological aspect of pigeon pea plants after 45 days of inoculation with bacterial isolates from the three sites (A : Kaffrine, B : Nioro, C : Fatick and D : Control)

Table.3 Groups (RFLP profiles) of infective bacterial isolates in the sites

Sites	Bacterial isolates	Groups (RFLP profiles)
FATICK	**F3p; F8p**	G1
	F11p	G7
	F9p	G8
	F7p	G9
	F12p	G5
NIORO	**N23; N24**	G1
	N19; N22	G3
	N3	G4
	N5; N10	G5
	N9	G6
	N8	G7
	N18	G8
KAFFRINE	**K6p**	G2
	K12p; K3	G3
	K13p	G4
	K16p	G5
	K4; K6	G6
	K9	G7
	K11	G8
	K16	G9
	K25	G10
	K18	G12

Table.4 Growth parameters of Pigeon pea Fatick provenance in Fatick soil after inoculation with specific bacterial isolates

With Fatick soil and seed provenance	SPAD	Nodule Number/plant	Nodule dry biomass/plant (mg)	Shoot dry biomass (mg)	Root dry biomass (mg)
Control	6.33 a	0.00 **c**	0.00 b	82.36 a	40. 00 b
F3p (G1)	8.40 a	3.66 bc	0.44 b	47.40 a	50.43 ab
F8p (G1)	7.50 a	11.00 abc	2.70 b	68.56 a	50.86 ab
F7p (G9)	9.80 a	7.33 abc	2.16 b	72.03 a	**71.00 a**
F9p (G8)	14.70 a	7.33 abc	2.06 b	57.86 a	44.20 b
F11p (G7)	14.43 a	**15.00 ab**	1.60 b	57.66 a	41.60 b
F12p (G5)	16.40 a	**19.66 a**	**29.65 a**	62.23 a	49.90 ab

F: bacterial isolates from Fatick soil, G: Group RFLP
In column, numbers with the same letter were not significantly different according to the Fisher test at the 5% threshold

Table.5 Growth parameters of Pigeon pea Nioro provenance in Nioro soil after inoculation with specific bacteria isolates

With Nioro soil and seed provenance	SPAD	Nodule number/Plant	Nodule dry biomass/plant (mg)	Shoot dry biomass (mg)	Root dry biomass (mg)
Control	6.33 b	0 f	0.00 b	82.36 ab	40.00 a
N23 (G1)	**27.37 a**	9.99 def	6.10 b	60.03 b	48.53 a
N24 (G1)	16.70 ab	28.66 ab	3.90 b	11.43 c	30.00 a
N1 (G3)	15.10 ab	11cdef	8.19 b	92.20 ab	52.86 a
N22 (G3)	**25.66 a**	20.66 bcde	5.20 b	**100.83 a**	55.76 a
N19 (G3)	19.33 ab	27.66 ab	5.66 b	56.47 b	46.70 a
N3 (G4)	19.20 ab	26 abc	6.60 b	92.73 ab	47.13 a
N5 (G5)	**23.86 a**	23 bcd	**23.63a**	**108.53 a**	41.43 a
N10 (G5)	18.56 ab	**40.33 a**	6.30 b	84.90 ab	44.40 a
N18 (G8)	**20.20 a**	6.33 ef	2.30 b	73.83 ab	52.83 a
N9 (G6)	18.56 ab	9 def	3.35 b	56.33 b	55.23 a
N8 (G7)	17.96 ab	14.33bcdef	1.15 b	84.93 ab	56.30 a

N: Bacterial isolates from Nioro soil, G: Group RFLP

In column, numbers with the same letter were not significantly different according to the Fisher test at the 5% threshold,

Table.5 Growth parameters of Pigeon pea Nioro provenance in Nioro soil after inoculation with specific bacteria isolates

With Nioro soil and seed provenance	SPAD	Nodule number/Plant	Nodule dry biomass/plant (mg)	Shoot dry biomass (mg)	Root dry biomass (mg)
Control	6.33 b	0 f	0.00 b	82.36 ab	40.00 a
N23 (G1)	**27.37 a**	9.99 def	6.10 b	60.03 b	48.53 a
N24 (G1)	16.70 ab	28.66 ab	3.90 b	11.43 c	30.00 a
N1 (G3)	15.10 ab	11cdef	8.19 b	92.20 ab	52.86 a
N22 (G3)	**25.66 a**	20.66 bcde	5.20 b	**100.83 a**	55.76 a
N19 (G3)	19.33 ab	27.66 ab	5.66 b	56.47 b	46.70 a
N3 (G4)	19.20 ab	26 abc	6.60 b	92.73 ab	47.13 a
N5 (G5)	**23.86 a**	23 bcd	**23.63a**	**108.53 a**	41.43 a
N10 (G5)	18.56 ab	**40.33 a**	6.30 b	84.90 ab	44.40 a
N18 (G8)	**20.20 a**	6.33 ef	2.30 b	73.83 ab	52.83 a
N9 (G6)	18.56 ab	9 def	3.35 b	56.33 b	55.23 a
N8 (G7)	17.96 ab	14.33bcdef	1.15 b	84.93 ab	56.30 a

N: Bacterial isolates from Nioro soil, G: Group RFLP

In column, numbers with the same letter were not significantly different according to the Fisher test at the 5% threshold,

Table.6 Growth parameters of Pigeon pea Kaffrine provenance in Kaffrine soil after inoculation with specific bacteria isolates

With Kaffrine soil and seed provenance	SPAD	Nodule Number/plant	Nodule dry biomass/plant (mg)	Shoot dry biomass (mg)	Root dry biomass (mg)
Control	6.33 e	0.00 e	0 f	82.36 ab	33.33 b
K6p (G2)	**22.80 b**	9.99 cde	1.83 def	58.70 b	31.10 b
K3 (G3)	**35.63 a**	**29.00 a**	**9.55 a**	113.60 a	55.73 b
K12p (G3)	**23.56 b**	7.66 de	**4.46 bcde**	93.03 ab	36.53 b
K13p (G4)	**22.00 b**	**23.00 ab**	**6.40 abc**	95.50 ab	48.80 b
K16p (G5)	**34.10 a**	**14.00 bcd**	**4.50 bcde**	73.50 ab	41.30 b
K6 (G6)	10.96 cde	**23.00 ab**	3.10 cdef	70.00 ab	34.60 b
K4 (G6)	11.55 cde	**31.00 a**	**7.43 ab**	104.56 a	42.60 b
K9 (G7)	**21.16 b**	**22.00 abc**	**5.93 abcd**	98.60 ab	44.13 b
K11 (G8)	**15.53 bcd**	10.33 bcde	2.85 cdef	85.76 ab	57.93 b
K16 (G9)	**18.46 bc**	9.00 de	0.50 ef	73.63 ab	59.53 b
K25 (G10)	9.80 de	**13.66 bcd**	1.13 ef	58.13 b	**198.63 a**
K18 (G11)	**18.90 bc**	9.33 cde	3.63 bcdef	101.06 ab	48.40 b

Analysis of the symbiotic status of different inoculated bacterial strains

The table 4 resumed the results obtained after inoculation of pigeon pea seedings with bacterial isolates from Fatick soil. Analysis of the effect of inoculation on chlorophyll content did not revealed a significant difference ($p \leq 0.05$) between inoculated plants and the control ones. Regarding the number of nodules, the analysis showed a significant difference between plants inoculated with F12p and F3p compared to the control ones. The dry mass of nodules in plants inoculated with F12p was significantly greater than in the other plants.

Results of pigeon pea seedlings inoculated with selected bacterial isolates from Nioro soil (Table 5) on leaf chlorophyll content revealed a significant difference ($p \leq 0.05$) between seedlings inoculated with isolates N23, N22, N5 and N18 and the uninoculated controls. Regarding the number of nodules, the analysis revealed a significant difference between seedlings inoculated with N10 and those inoculated with N23, N22, N5, N18, N9, N8 and N1. The dry biomass of nodules in N5 seedlings was significantly higher than in the other ones. Seedlings inoculated with isolates N23, N22 and N5 exhibited significantly higher chlorophyll content compared to the other seedlings.

In Kaffrine soil, analysis of the effect of inoculation on chlorophyll content with different bacterial isolates revealed a significant difference ($p \leq 0.05$) between plants inoculated with isolates K3, K16p, K12p, K6p, K13p, K9, K18, K16 and K11, and the control plants (Table 6). Regarding the number of nodules, the analysis showed a significant difference between plants inoculated with K3 and K4 and those inoculated with K16p, K12p, K6p, K18, K16, K11 and K25. The dry biomass of nodules in plants inoculated with K3 was significantly higher than in plants inoculated with K16p, K12p, K6p, K18, K16, K11 and K25. Plants inoculated with K3 and K16p isolates have higher chlorophyll content compared to other plants.

Correlations between growth parameters and bacterial isolates in each site

In each soil conditions, a principal component analysis (PCA) was performed on five growth and symbiotic variables. In Fatick soil, the PCA showed that the first two axes explained 73.38% of the total variability (Figure 3). These two first axes highlighted a strong differentiation among treatments, mainly driven by nodulation parameters. Treatment F12p (G5) was clearly associated with high nodule number and nodule weight,

while F7p (G9) was distinguished by high root dry biomass. The control and F3p (G1) treatments were characterized by low nodulation and clustered separately from high-performing treatments. In Nioro soil, the two first axes of the PCA explaining 67.08% of the variability (Figure 4). This later revealed a clear separation of treatments based on nodulation and biomass parameters. Treatments N5 (G5), N10 (G5) and N22 (G3) were associated with high nodule number, nodule weight and shoot dry biomass, whereas the control and N24 (G1) were characterized by low values for these variables. Root dry biomass showed a more moderate contribution to the structuring of the PCA space. In Kaffrine soil, the PCA encompassed 76.44% of the total variability (Figure 5). Axis F1 (55.31%) was positively correlated with SPAD value, nodule number, nodule weight and shoot dry biomass, representing symbiotic efficiency and plant vigor. Axis F2 (21.13%) was mainly associated with root dry biomass, reflecting differences in biomass allocation strategies. Treatments K3 (G3), K4 (G6) and K9 (G7) were strongly associated with high symbiotic performance, while the control and K6p (G2) showed poor growth and nodulation. K25 (G10) was clearly separated along F2 due to its exceptionally high root dry biomass, indicating an atypical response.

The discussion of this study includes, to our knowledge, this work is the first to address the diversity of rhizobial communities associated with pigeon pea plant in Senegal. Different results were obtained regarding soil and seed provenances.

Most Problable Number of rhizobia in soils

Nitrogen-fixing symbionts play an important role in regulating the productivity of plant and terrestrial ecosystems through a mutualistic symbiosis with legumes (Jaiswal and Dakora, 2025; Yeremko *et al*., 2025). The number of bacteria per gram of dry soil obtained differed between the soil sites. The bacteria populations in the soils of Kaffrine and Fatick were higher than those in the soil of Nioro. The soils are heterogeneous mixtures of various components, including organic matter, silt, sand, clay and inorganic salts that could influence microbial survival (Zhang *et al*., 2023). The acidity level observed in the three soils is close to neutrality and is favorable for the presence and survival of microorganisms. However, there is no significant difference in pH between the three soils. Exchangeable bases (Ca^{2+}, Mg^{2+}, Na^{+}), nitrogen (N), and phosphorus (P) concentrations are higher in the soil of Nioro where the lowest bacterial population is recorded. This result is consistent with the findings of Alon *et al*. (2021), which showed low nodulation in soils with high nutrient concentrations. Under these conditions, bacteria produce fewer root nodules, as they receive fewer signals from the plant to initiate this symbiotic interaction. The greater abundance and variation of rhizobia in the soils might also be due to the presence of non-leguminous plants in soils with high rhizobium populations, as these non-leguminous plants help rhizobia maintain a higher population level (Kebede *et al*., 2021).

Phenotypic diversity of bacterial isolates

On YMA culture medium, rhizobia are subdivided into two groups. This medium is used to evaluate bacterial growth rate by measuring the time it takes for complete colonies to form. The first group consists of bacteria probably belonging to the genus *Rhizobium*, with a complete colony formation time of two days or less, referred to as "fast-growing" bacteria. The second group consists of bacteria probably belonging to the genus *Bradyrhizobium*, referred to as "slow-growing" bacteria, characterized by the development of complete colonies between three and five days of incubation. Previous studies have confirmed that fast-growing bacteria form visible colonies on YMA culture medium within 1 to 3 days of incubation, while slow-growing bacteria take 3 to 5 days to form visible colonies under the same conditions (Kebede *et al*., 2022).

In the present study, at all three sites, the isolated bacteria were both fast- and slow-growing, with a significantly higher percentage of fast-growing bacteria. In fact, 91% of the isolated bacteria were fast-growing bacteria. This result corroborated previous works and confirmed the findings of Chalasani *et al*. (2021) and Jorrin *et al*. (2021), who reported that although pigeon pea preferential symbionts are *Bradyrhizobium* spp., these symbionts exhibit low competitiveness with respect to *Rhizobium* spp., and that pigeon pea roots have reduced microbial diversity compared to the surrounding soil and select more for *Rhizobium* spp. during vegetative growth. This dominance of fast-growing bacteria over slow-growing ones was observed in all three soil types. Similarly, other morphological characteristics like diameter, shape, surface area, consistency, color, were not site-specific and were identical across all or many groups.

ITS 16S-23S rDNA genetic diversity of nodule bacterial isolates

Analysis of genetic diversity obtained by PCR-RFLP on the 16S-23S intergenic region rDNA revealed 28 distinct genetic profiles among the 87 isolates. These results suggest significant diversity in rhizobia associated with pigeon pea in Senegal. The genetic profiles varied according to the site, with greater diversity in Kaffrine and Fatick. This disparity could be linked to a higher microbial density in these soils and to the presence of biotic and abiotic factors influencing the composition of symbiotic populations. This confirms the importance of the 16S-23S rDNA genetic marker, that allows a fine differentiation of isolates based on their genetic heterogeneity in the study of bacterial diversity in general, and rhizobial diversity in particular. The observed inequality in the nodulation frequencies of genetic groups across the three study sites can be explained by the non-identical competitive capacity of the groups at each site during the nodulation process and by unequal saprophytic population density among the different rhizobial strains present in the pigeon pea rhizosphere (Fossou, 2011). This phenomenon could also be explained by the result of interactions between rhizobia, their leguminous hosts, and the biotic and abiotic factors of the ecosystem. Indeed, some strains maintain a low population density in the soil, in the absence of the host-legume (Geremu *et al*., 2025). Sites with a higher initial population density (Kaffrine and Fatick) naturally have more potential strain candidates for nodulation and therefore greater genetic diversity.

Infectivity and effectivity of nodule bacterial isolates

The effectiveness of a symbiotic relationship (Rhizobium-plant) is characterized by the ability of the bacterium to positively impact the development and growth of the host plant. Results revealed a significant positive effect of inoculation with certain bacterial isolates on growth parameters (Nodule number and dry biomass, shoot and root dry biomass, shoot chlorophyll content) of pigeon pea in each soil. It also appears that nodulation and nodulation efficiency in pigeon pea are particularly dependent on the inoculated strain. According to Zhao *et al*. (2025), chlorophyll is an indicator of the degree of nitrogen assimilation and is responsible for the green coloration of leaves. In this study, bacterial named isolates N23, N22, N5, K3 and K16p likely induced relatively high chlorophyll levels in the leaves of pigeon pea plants compared to the control ones. According to Woomer (2010), high chlorophyll levels are associated with good plant colonization and could represent an indicator of the effectiveness of the inoculated strain. In this study, the rate of increase in chlorophyll content is much greater in plants inoculated with fast-growing bacteria. In these plants, the content is higher during the first two weeks, then decreased before increasing again (data not shown). This phenomenon could be explained by the fact that during the first two weeks, the plants use the nitrogen reserves stored in the cotyledons. After these, reserves are depleted, the content increases in plants inoculated with the most efficient nitrogen-fixing bacteria, while it decreases in plants inoculated with non-nitrogen-fixing or inefficient bacteria. However, this high chlorophyll content was not accompanied by a significant number of nodules or nodule dry biomass. Furthermore, more than half of the isolates did not exhibit an ability to increase leaf chlorophyll content. Regarding shoot and root dry biomasses, plants inoculated with the bacterial isolates K25 and F7 produced significantly greater root biomass than the control ones. Moreover, the N5 plants produced higher aboveground biomass compared to the control plants. This result corroborated that of Andrade *et al*. (2019), who found a positive effect of rhizobial inoculation on shoot and root biomasses compared to the uninoculated control. Bacterial isolates named N10, K4, F12p and F11p produced significant numbers of nodules but did not show a significant impact on chlorophyll content. This result suggests that massive nodulation does not always indicate the symbiotic efficiency of a rhizobium strain, particularly in its ability to improve plant growth and productivity (Sene *et al*., 2023). Growing conditions that could stress the plants could help explain this result.

Revellin (2012) defined the infectivity of a rhizobium strain as nodule formation and its efficiency as nitrogen fixation *via* leghemoglobin. A strain can be infectious by forming nodules that are ineffective in biological nitrogen fixation (Basile & Lepek, 2021). At the Fatick site, the chlorophyll content of inoculated plants was not different from that of uninoculated ones. This suggests that these inoculated isolates have the capacity to infect their host but are not effective. This result showed that the infectivity of a strain does not always justify its symbiotic efficiency (Kaziūnienė *et al*., 2025); and that some pigeon pea varieties have a low probability of

symbiotic potential. Chalasani *et al*. (2021) hypothesized that "the low efficiency of reported pigeon pea nodulation is the result of either the low number of compatible symbionts in the soil or their low competitiveness in colonizing the host-plant."Nodulation alone does not always lead to an efficient symbiosis capable of meeting the nitrogen requirements of the host-plant (Allito *et al*., 2021). This requires extensive exploration and selection of elite rhizobial symbionts that can efficiently infect leguminous hosts and, thus, fix a significant amount of atmospheric nitrogen (Woliy *et al*., 2019).

Relationship between growth and symbiotic variables in the three soil sites

From PCA analysis, rate of variability in Fatick, Nioro and Kaffrine soils, respectively, are strong for an excellent interpretation. Analysis underlined a key relationship on PCA1 with a high synergy between nodules and biomass variables; and a moderate synergy between nodule dry weight and biomass. It indicates that the soil/plant context radically modulates symbiotic efficiency (nodule conversion to growth). In PCA2, SPAD is very independent but linked to nodules. The physiological response (chlorophyll) is a distinct axis of variation, more or less coupled to symbiosis depending on the site. Among all bacterial strain tested, F12p (G5) and K3 (G3) were the best and the "Star" strains. The bacterial strain N5 (G5) is exceptional with nodule weight. It appeared a site-specific performance because no strain/group is universally better. Control treatment is separated from the others, translating its low performance. It indicated that the need for inoculation is evident in Fatick and Kaffrine, but less so in Nioro.

From the analysis, the most important finding is that there is no universal bacterial strain and that the performance of a bacterial strain is highly dependent on the tripartite interaction between soil, host-plant and rhizobium. It has also emerged three models of symbiotic efficiency with in Fatick a strong synergy (inoculation results in overall better plants). In Nioro, competition/Trade-off domine indicated that investment in nodulation can come at the expense of shoot biomass. At least, in Kaffrine, a moderate synergy and decoupling is detected, underlining that growth and chlorophyll production respond more independently.

In conclusion, this study highlighted the variability in the density and diversity of rhizobia associated to pigeon pea in three regions of the Senegalese groundnut basin. The results revealed that the soils of Kaffrine and Fatick have higher rhizobial populations than that of Nioro. The bacterial isolates exhibit phenotypic diversity, with a dominance of fast-growing strains, and genetic diversity was observed between sites. Some bacterial isolates showed a positive effect on nodulation, chlorophyll content and pigeon pea growth. The moste suitable bacterial strains are F12p for Fatick, N23, N22 and N5 for Nioro, and at least, K3 and K16p for kaffrine.
The genetic identification of the strains will be further examined, and their symbiotic efficiency will be tested under field conditions. Promising rhizobial isolates could be used to develop biofertilizers adapted to pigeon pea. Promoting the integration of this legume into local agricultural systems could also contribute to improving soil fertility and food security.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgement

The authors are grateful to the LMI-LAPSE (Laboratoire mixte international Adaptation des Plantes et microorganismes associés aux Stress Environnementaux, IRD) for financial support. They also thank the Women Association of the farm in Kaffrine, the Owner of Kaydara farm in Fatick and ANCAR (Agence Nationale de Conseil Agricole et Rural) of Nioro for efficient collaborations.

Author contributions

F. Ndoye : Investigation, analysis, writing original draft, T. Krasova-Wade: Methodology, student supervising, writing-reviewing, A. Kane: Conceptualization, methodology, writing and funding acquisition, S. Ngom: Investigation, data collection and analysis, editing, D. Niang: Student supervision, methodology, statistical analysis, reviewing, P.T.G. Sane: Data collection and analysis, C.V. Nadieline: Student supervision and methodology, M. Ngom: Conceptualization and reviewing, C. Ndiaye: Soil and seed sampling and methodology, D. Diouf: Conceptualization, funding acquisition and reviewing, M.O. Sy: Conceptualization, validation and writing-reviewing, A.G. Diedhiou:

Conceptualization, investigation, protocol validation and supervision

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.
Conflict of Interest The authors declare no competing interests.

References

Allito B.B., Ewusi-Mensah N., Logah V. *et al.* (2021). Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (*Vicia faba* L.). *Scientific Reports,* 11, 3678. https://doi.org/10.1038/s41598-021-83235-8

Alon M., Dovrat G., Masci, T. & Sheffer, E. (2021). Soil nitrogen regulates symbiotic nitrogen fixation in a legume shrub but does not accumulate under it. *Ecosphere*, 12(12), e03843. https://doi.org/10.1002/ecs2.3843

Baldermann S., Blagojević L., Frede K., Klopsch R., Neugart S., Neumann A., Ngwene B., Norkeweit J., Schröter D., Schröter A., Schweigert F.J., Wiesner M. & Schreiner M. (2016). Are Neglected Plants the Food for the Future? *Critical Reviews in Plant Sciences*, 35(2), 106-119. https://doi.org/10.1080/07352689.2016.1201399

Basile LA & Lepek VC. 2021. Legume-rhizobium dance: an agricultural tool that could be improved? Microb Biotechnol. 2021 Sep;14(5):1897-1917. https://doi.org/10.1111/1751-7915.13906

Chalasani D., Basu A., Pullabhotla S.V.S.R.N., Jorrin B., Neal A.L., Poole P.S., Podile A.R. & Tkacz, A. (2021). Poor Competitiveness of *Bradyrhizobium* in Pigeon Pea Root Colonization in Indian Soils. *mBio*, *12*(4), e00423-21. https://doi.org/10.1128/mbio.00423-21

de Andrade F.M., de Assis Pereira T., Souza T.P., Guimarães P.H.S., Martins A.D., Schwan R.F., Pasqual M. & Dória, J. (2019). Beneficial effects of inoculation of growth-promoting bacteria in strawberry. *Microbiological research*, 223, 120-128. https://doi.org/10.1016/j.micres.2019.04.005

Diedhiou I., Diedhiou A.G., & Diouf, D. (2022). Les symbioses fixatrices d'azote : Types et régulateurs transcriptionnels de la nodulation. *International Journal of Biological and Chemical Sciences*, 16(2), 695-712. http://ajol.info/index.php/ijbcs

Duguma H.T. (2020). Wild Edible Plant Nutritional Contribution and Consumer Perception in Ethiopia. *International Journal of Food Science*, 1-16. https://doi.org/10.1155/2020/2958623

Falola A., Mukaila R., Lawal T.F. & Akinsuyi M. (2022). Commercialization of pigeon pea production : Its determinants and constraints. *Tekirdağ Ziraat Fakültesi Dergisi*, 19(4), 840-849. https://doi.org/10.33462/jotaf.1113523

Ferreira I. C., Morales P. & Barros L. (2017). *Wild plants, mushrooms and nuts : Functional food properties and applications*. John Wiley & Sons, 496 pages.

Fisher, R. A. & Yates F. (1963). *Statistical tables for biological, agricultural and medical research, edited by R. fisher and F. Yates*. Edinburgh: Oliver and Boyd. *Biometrical journal fmi, 225-286.*

FAO, IFAD, UNICEF, WFP and WHO (2025). *The State of Food Security and Nutrition in the World 2025 – Addressing high food price inflation for food security and nutrition*. Rome. https://doi.org/10.4060/cd6008en

Fossou K.R. (2011). Diversité génétique des rhizobia associés à un champ de pois d'Angole (*Cajanus cajan* L.) à Yamoussoukro (Centre de la Côte d'Ivoire). *Mémoire de fin d'études pour l'obtention du diplôme d'Agronomie Approfondie, Ecole Supérieure d'Agronomie de Yamoussoukro (Côte d'Ivoire)*, *63*.

Fossou R.K., Kouassi N., Kouadjo G.C.Z., Zako S. & Zeze A. (2012). Diversité de rhizobia dans un champ cultivé de pois d'angole (*Cajanus cajan* L.) (légumineuses) à Yamoussoukro (centre Côte d'Ivoire). *Agronomie Africaine*, 24(1), 29-38.

Fatokimi E. & Tanimonure V.A. (2021). Analysis of the current situation and future outlooks for pigeon pea (*Cajanus cajan*) production in Oyo State, Nigeria: A Markov Chain model approach. *Journal of Agriculture and Food Research* 6(2):100218. https://doi.org/10.1016/j.jafr.2021.100218

Faye A., Stewart Z.P., Diome K., Edward C.T., Fall D., Ganyo D.K.K., Akplo T.M. & Prasad, P.V.V. (2021). Single Application of Biochar Increases Fertilizer Efficiency, C Sequestration, and pH over the Long-Term in Sandy Soils of Senegal. *Sustainability*, 13 (21), 11817.

https://doi.org/10.3390/su132111817

Geremu T., Abera G., Lemma B. & Rasche F. (2025). Abundance and symbiotic efficiency of indigenous rhizobia nodulating faba bean and common bean in southern Ethiopia. *Frontiers in Soil Sciences*, 5:1568292. https://doi.org/10.3389/fsoil.2025.1568292

Jaiswal S. K. & Dakora F. D. (2025). Maximizing Photosynthesis and Plant Growth in African Legumes Through Rhizobial Partnerships: The Road Behind and Ahead. *Microorganisms*, 13(3), 581. https://doi.org/10.3390/microorganisms13030581

Jeevarathinam G. & Chelladurai V. (2020). Pigeon Pea. In *A. Manickavasagan et P. Thirunathan (Éds.), Pulses* (p. 275-296). Springer International Publishing

Jorrin B., Maluk M., Atoliya N., Kumar S.C., Chalasani D., Tkacz A., Singh P., Basu A., Pullabhotla S. V. & Kumar M. (2021). Genomic diversity of pigeon pea (Cajanus cajan L. Millsp.) endosymbionts in India and selection of potential strains for use as agricultural inoculants. *Frontiers in plant science*, *12*, 680981. https://doi.org/10.3389/fpls.2021.680981

Kaziūnienė J., Gegeckas A., Lapinskienė L., Razbadauskienė K., Mažylytė R. & Supronienė S. (2025). Competitiveness and Nitrogen Fixation Efficiency Analysis of *Rhizobium leguminosarum* Strains in Different Field Pea (*Pisum sativum* L.) Genotypes. *Agriculture*, 15(16), 1784. https://doi.org/10.3390/agriculture15161784

Kebede E., Amsalu B., Argaw A. & Tamiru S. (2022). Nodulation potential and phenotypic diversity of rhizobia nodulating cowpea isolated from major growing areas of Ethiopia. *Agrosystems, Geosciences et Environment*, *5*(4), e20308. https://doi.org/10.1002/agg2.20308

Krasova-Wade T., Ndoye I., Braconnier S., Sarr B., de Lajudie P. & Neyra. M. 2003. Diversity of indigeneous bradyrhizobia associated with three cowpea cultivars (*Vigna unguiculata* (L.) Walp.) grow under limited and favorable water conditions in Senegal (West Africa). *African Journal of Biotechnology*, 2(1):13-22. https://doi.org/10.5897/AJB2003.000-1003

Khuntia D., Pand N., Mandal M., Swain P., Sahu S.G. & Pattanayak S.K. (2022). Symbiotic Effectiveness of Acid Tolerant Nodulating *Rhizobia* on Growth, Yield and Nutrient Uptake of Pigeon pea (*Cajanus cajan* L.) in Acidic Alfisols. *International Journal of Bio-resource and Stress Management,* 13(4), 403-410. https://doi.org/10.23910/1.2022.2550

Lam D. (2025). The Next 2 Billion: Can the World Support 10 Billion People?. *Population and Development Review*, 51: 63-102. https://doi.org/10.1111/padr.12685

Mathieu C. & Pieltain F. 2003. *Analyse Chimique des Sols*: *Méthodes Choisies*; *Edition Tec & Doc:* Montpellier, France, 387p.

Namuyiga D.B., Stellmacher T., Borgemeister C. & Groot J.C.J. (2022). A Typology and Preferences for Pigeon Pea in Smallholder Mixed Farming Systems in Uganda. *Agriculture*, 12(8), 1186. https://doi.org/10.3390/agriculture12081186

Normand P., Ponsonnet C., Nesme X., Neyra M. Simonet P. (1996). *ITS analysis of prokaryotes. Molecular Microbial Ecology Manual (Akkermans DL, van Elsas JD et de Bruijn EI, eds)*. Kluwer Academic Publishers, Amsterdam.

Pazhamala L., Saxena R.K., Singh V.K., Sameerkumar C.V., Kumar V., Sinha P., Patel K., Obala J., Kaoneka S.R. & Tongoona P. (2015). Genomics-assisted breeding for boosting crop improvement in pigeonpea (*Cajanus cajan*). *Frontiers in plant science*, *6*, 50. https://doi.org/10.3389/fpls.2015.00050

Revellin, C. (2012). Les symbioses fixatrices d'azote. *Alliances au pays des racines-14 e Colloque Scientifique de la Société d'Horticulture de France, Paris, FRANCE*.

Sane C.A.B., Rouland-Lefevre C., Grechi I., Rey J.Y., Vayssieres J.F., Diame L. & Diarra K. 2016. Diversité, nuisances et modes de gestion des termites (Isoptera) dans les agrosystèmes sénégalais. *International Journal of Biological and Chemical Sciences*, 10(1): 134-154, http://dx.doi.org/10.4314/ijbcs.v10i1.10

Sene G., Ndiaye B.D., Cissoko M., Niang N., Ndiaye C., Fall S. & Sylla S.N. (2023). Growth promotion and yield attribute improvement of five groundnut (*Arachis hypogaea* L.) varieties by the application of plant growth promoting rhizobacteria. *African Journal of Agricultural Research,* 19(12): 1151-1160. http://dx.doi.org/10.5897/AJAR2023.16553

Somasegaran P. and Hoben H.J. (1994). Quantifying the Growth of Rhizobia. In P. Somasegaran and H. J. Hoben, *Handbook for Rhizobia* (p. 47-57). Springer New York.

Sukati M. (2020). National Income and Malnutrition in Africa: A Rapid Overview. *Advances in Pediatrics & Neonatal care*, 113.

https://doi.org/10.29011/APNC-113.100013

UNSCN. 2020 – The COVID-19 pandemic is disrupting people's food environments: a resource list on Food Systems and Nutrition responses-UNSCN. Unscn.org

Vincent J.M. (1970). A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria.*

Woliy K., Degefu T. & Frostegård Å. (2019). Host range and symbiotic effectiveness of N2O reducing *Bradyrhizobium* strains. *Frontiers in Microbiology*, 10, 2746. https://doi.org/10.3389/fmicb.2019.02746

Woomer P.L. (2010). Biological nitrogen fixation and grain legume enterprise : Guidelines for N2 Africa lead farmers. *Tropical soil biology and fertility institute of the international centre for tropical agriculture. Nairobi/International Institute of Tropical Agriculture, Ibadan Nigeria*, *21*.

Yeremko, L., Czopek, K., Staniak, M., Marenych, M., & Hanhur, V. (2025). Role of Environmental Factors in Legume-*Rhizobium* Symbiosis: A Review. *Biomolecules*, *15*(1), 118. https://doi.org/10.3390/biom15010118

Zhang Z.Y., Qiang F.F., Liu G.Q., Liu C.H. & Ai N. (2023). Distribution characteristics of soil microbial communities and their responses to environmental factors in the sea buckthorn forest in the water-wind erosion crisscross region. *Frontiers in Microbiology,* 13:1098952. https://doi.org/10.3389/fmicb.2022.1098952

Zhao J., Gao A., Wang B., Wen J., Duan Y., Wang G. & Li Z. (2025). Modeling and Visualization of Nitrogen and Chlorophyll in Greenhouse *Solanum lycopersicum* L. Leaves with Hyperspectral Imaging for Nitrogen Stress Diagnosis. *Plants* (Basel), 14(21):3276. https://doi.org/10.3390/plants14213276

How to cite this article:

Fatou NDOYE, Tatiana Krasova-WADE, Aboubacry KANE, Diariatou NIANG, Seynabou NGOM, Papa Talybe Gueye SANE, Christian Valentin NADIEDINE, Mariama NGOM, Cheikh NDIAYE, Diegane DIOUF, Mame Oureye SY, Abdala Gamby DIEDHIOU. 2026. Characterization of Bacterial Rhizobial Communities Associated to three Provenances of Pigeon Pea (*Cajanus cajan* L.) Cultivated in Soils from three Different Regions in Senegal. *Int.J.Curr.Microbiol.App.Sci.* 15(1): 1-17. **doi:** https://doi.org/10.20546/ijcmas.2026.1501.001