MAJOLO, C.; DOS SANTOS, J. P.; PERIN, R.; RIBEIRO, N. H. S.; DE ABREU, J. A. A.; SANTOS, S. F.; DA SILVA, E. L.; REIS, V. R.; NOGUEIRA, M. A.; MUNIZ, A. W. Characterization and selection of native rhizobia from amazonian soils for *Vigna unguiculata* (l.) Walp. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 13, n. 1, e9495, 2026. ISSN 2358-6303. DOI: https://doi.org/10.32404/rean.v13i1.9495.

Characterization and selection of native rhizobia from amazonian soils for *Vigna unguiculata* (l.) Walp.

Cláudia Majolo[1], Jéssica Pinheiro dos Santos[1], Rogério Perin[1], Natasha Helena Souza Ribeiro[1], July Anne Amaral de Abreu[1], Samára Ferreira Santos[1], Elen Lira da Silva[1], Vanessa Ribeiro Reis[1], Marco Antônio Nogueira[2], Aleksander Westphal Muniz[1]

[1] Embrapa Amazônia Ocidental, Manaus, Amazonas, Brasil. E-mail: claudia.majolo@embrapa.br, jessicabrasillps02@gmail.com, rogerio.perin@embrapa.br, natashahelenaribeiro@gmail.com, july.abreu@ufv.br, samara.baggins@gmail.com, liraelen519@gmail.com, ribeiro_vanessar@hotmail.com, aleksander.muniz@embrapa.br

[2] Embrapa Soja, Londrina, Paraná, Brasil. E-mail: marco.nogueira@embrapa.br

Received: 04/16/2025; Accepted: 02/14/2026.

ABSTRACT

Cowpea (*Vigna unguiculata*) is a legume of great socioeconomic importance in Brazil, particularly in the North and Northeast regions, where it accounts for about 30% of the country's total bean production. It plays a critical role in food security and income generation for smallholder farmers, especially in challenging environments such as the Amazon. However, cowpea yield remains limited due to low technology adoption and poor management practices. Biological nitrogen fixation (BNF), mediated by symbiotic bacteria such as *Bradyrhizobium* and *Rhizobium*, offers a sustainable alternative to chemical fertilizers. This study aimed to isolate and characterize rhizobia from distinct Amazonian soils (Floodplain, Amazonian dark earth, and Oxisol). The isolates were evaluated in greenhouse conditions for cowpea growth promotion and characterized by 16S rRNA gene sequencing. Shoot dry mass (SDM) ranged from 736.67 to 2,220 mg per plant, and shoot dry mass nitrogen (SDMN) from 17.09 to 66.85 mg per plant. Most isolates promoted nodulation, with *Rhizobium* predominating, and several isolates showed similar agronomic performance, not statistically different from the recommended strain SEMIA 6462. The results indicate the potential of these native isolates for use as bioinoculants adapted to Amazonian conditions. These findings highlight the potential of native rhizobia as candidates for the development of bioinoculants adapted to Amazonian conditions.

Keywords: Cowpea, Biological nitrogen fixation, *Bradyrhizobium*, *Rhizobium*, Amazon.

Caracterização e seleção de rizóbios nativos de solos amazônicos para *Vigna unguiculata* (l.) Walp

RESUMO

O feijão-caupi (*Vigna unguiculata*) é uma leguminosa de grande importância socioeconômica no Brasil, especialmente nas regiões Norte e Nordeste, onde responde por cerca de 30% da produção nacional de feijão. Sua relevância está associada à segurança alimentar e à geração de renda para pequenos produtores, sobretudo em ambientes de cultivo adversos, como os da Amazônia. Entretanto, sua produtividade é limitada pelo baixo uso de tecnologias e manejo inadequado. A fixação biológica de nitrogênio (FBN), mediada por bactérias simbióticas como *Bradyrhizobium* e *Rhizobium*, constitui uma alternativa sustentável aos fertilizantes químicos. Este estudo teve como objetivo isolar e caracterizar rizóbios oriundos de solos amazônicos distintos (várzea, terra preta de índio e latossolo). As bactérias isoladas foram avaliadas em casa de vegetação quanto à promoção de crescimento do caupi e caracterizadas por sequenciamento do gene 16S rRNA. A massa seca da parte aérea (MSPA) variou de 736,67 a 2.220 mg por planta, e o acúmulo de nitrogênio (N-MSPA), de 17,09 a 66,85 mg por planta. A maioria dos isolados promoveu nodulação, com predominância do gênero *Rhizobium*, e vários isolados apresentaram desempenho agronômico semelhante, não diferindo estatisticamente da estirpe recomendada SEMIA 6462. Os resultados indicam o potencial desses rizóbios nativos como candidatos para o desenvolvimento de bioinoculantes

adaptados às condições amazônicas.

Palavras-chave: Feijão-caupi, Fixação Biológica de Nitrogênio, *Bradyrhizobium*, *Rhizobium*, Amazônia.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.), also known as cowpea bean, is a legume of great socioeconomic importance in Brazil, particularly in the North and Northeast regions. These areas dominate its production, accounting for approximately 30% of the country's total bean production, while common beans (*Phaseolus vulgaris*) make up the remaining 70% (Bertine et al., 2008; Filgueiras et al., 2009). In addition to being a vital source of protein and energy in these regions, cowpea has a remarkable ability to adapt to adverse environmental conditions, such as low water availability, high temperatures, and low-fertility soils, which are frequently found in Amazonian ecosystems (Shevkani et al., 2025; Oliveira et al., 2019).

In the state of Amazonas, cowpea is widely consumed in local cuisine, especially in traditional dishes like 'baião de dois', and plays an essential role in ensuring food security for rural communities. Additionally, its cultivation is an important source of income for small farmers, providing a viable economic alternative even under adverse agricultural conditions (CONAB, 2024).

Despite cowpea's regional importance, its yield in the Amazon and other parts of Brazil falls short of its potential. Several factors contribute to this limitation, including the low adoption of appropriate agricultural technologies, inadequate management of inputs, and a lack of knowledge about practices that could enhance crop yields (Silva et al., 2010). In this context, developing sustainable approaches adapted to local conditions, such as the use of rhizobia, emerges as a promising strategy to improve agricultural efficiency and reduce reliance on chemical fertilizers (Hungria et al., 2007).

Biological nitrogen fixation (BNF) is a natural process in which symbiotic bacteria, such as those from the *Bradyrhizobium* and *Rhizobium* genera, convert atmospheric nitrogen into plant-available forms such as ammonia. This process is crucial for legume crops like cowpea, which have a high capacity to form root nodules where this symbiosis with bacteria occurs (Moreira and Siqueira, 2006). Inoculation with rhizobia can significantly increase soil nitrogen availability, thereby promoting plant growth and yield.

Studies conducted in various parts of the world, particularly in tropical soils of Africa and Latin America, have demonstrated that inoculation of legumes with rhizobia can increase both biomass production and grain yield. These findings highlight the great potential of applying these techniques in tropical and subtropical regions, where soils are typically nutrient-poor, and agriculture faces sustainability challenges (Kan et al., 2007; Almeida Neta et al., 2020; Messias et al., 2023).

In the Amazon, where soil diversity is remarkable, including fertile Terra Preta de Índio (Amazonian Dark Earth), floodplain soils, and Oxisols, biological nitrogen fixation becomes even more important. These soils have markedly different chemical and physical characteristics, which directly influence nutrient availability and crop development. For example, floodplain soils naturally have higher fertility due to sediment deposition, while Oxisols are notoriously nutrient-poor and require more intensive management strategies (Schellekens et al., 2017; Junk et al., 2020).

Despite the potential for using BNF in the Amazon, knowledge of native strains of these bacteria and their effectiveness across different soil types remains limited. This is partly due to a lack of research focused on identifying and characterizing these strains, which could serve as bioinoculants adapted to local edaphoclimatic conditions. Characterizing efficient native BNF strains from the Amazon can help develop biotechnological products that enhance agricultural productivity and promote more sustainable agriculture in the region.

Given this scenario, there is a growing need for studies to isolate, identify, and test the efficacy of native BNF strains in Amazonian soils, focusing on their ability to promote plant growth and fix nitrogen. A better understanding of microbial diversity across different Amazonian soil types will enable the development of more effective agricultural strategies that integrate low-input practices and promote the sustainability of agricultural production in the Amazon.

Thus, the objective of this study was to isolate and characterize BNF strains from cowpea plants cultivated in different Amazonian soils to assess their potential for in vitro plant growth promotion, and to evaluate their growth-promoting capacity in sterile substrate in greenhouse conditions. Additionally, the 16S rRNA gene of the most promising strains was sequenced to assess the genetic diversity of these bacteria. The selected strains in this study may serve as candidates for future field applications, requiring longer-term efforts to produce agriculturally relevant bioinoculants.

2. Material and Methods

Soil samples were collected in the 0-10 cm layer in three distinct environments: Terra Preta de Índio/Amazonian Dark Earth; Terra de Várzea/Floodplain Soil, and Latossolo Amarelo/Oxisol

in the municipalities of Iranduba and Manaus, AM. After collection, composite samples from each site were subjected to particle-size analysis using the pipette method and to soil fertility analysis (Table 1).

Table 1. Chemical characterization of the soils sampled for rhizobial nodule collection

Soil	Location	pH	C	O.M	P	K	Na	Ca	Mg	Al	H+Al	SB	t	T	V	m
			g kg^{-1}		mg dm^{-3}			cmolc dm^{-3}							%	
ADE	3°15'10.03"S 60°13'42.10"W Iranduba/AM	5.63	43.49	74.80	91	38	7	7.30	1.27	0.00	4.21	8.70	8.70	12.91	67.40	0,00
FP	3°15'36.3"S 60°13'22.4"W Iranduba/AM	5.89	7.14	12.28	64	87	38	6.68	2.32	0.00	2.81	9.39	9.39	12.19	76.99	0,00
OX	2°53'40.8"S 59°59'21.3"W Manaus/AM	4.16	35.35	60.80	6.1	36	5	0.32	0.11	1,00	2.21	0.54	1.54	2.76	19.7	64.78

ADE: Amazonian Dark Earth; FP: Floodplain Soil; OX: Oxisol; pH in water - 1:2.5 ratio; P, Na, K, - Mehlich-1 Extractor; Ca, Mg - 1 mol/L KCl Extractor; H+Al - 0.5 mol/L Calcium Acetate Extractor - pH 7.0; SB - Sum of Exchangeable Bases; CEC (t) - Effective Cation Exchange Capacity; CEC (T) - Cation Exchange Capacity at pH 7.0; V - Base Saturation Index; m - Aluminum Saturation Index; Organic Matter (O.M) = C (organic carbon) x 1.724 - Walkley-Black

These soil samples were used to establish trap cultures using cowpea (*Vigna unguiculata*) as the host plant. The collected soil was gently homogenized and placed in 500 mL plastic containers, in which cowpea seeds were sown. After 45 days of growth, the plants were harvested, and root nodules were collected for the isolation of nitrogen-fixing bacteria.

The collected nodules were disinfected with a 70% alcohol-sodium hypochlorite solution, as described by Hungria and Araújo (1994). Subsequently, using sterilized forceps and a platinum loop, the nodules were inoculated onto Petri dishes containing Yeast-Mannitol Agar (YMA) medium. The plates were incubated in a bacteriological incubator at 28°C for a period of three to 28 days, with daily monitoring to observe rhizobial growth and potential contaminants.

The isolates' phenotypic characterization was conducted by observing morphological characteristics and biochemical tests. The morphological characteristics evaluated included color, consistency, and colony shape. Biochemical tests assessed catalase production, oxidase activity, Gram staining, indoleacetic acid production, and calcium phosphate solubilization (Muniz and Majolo, 2024).

The elite isolates (n=22) used in the greenhouse experiment were previously selected from earlier experiments (Supplement). This experiment was conducted in a completely randomized design with four replications. Cowpea (*Vigna unguiculata*) was cultivated in pots containing a sterile mixture of sand and vermiculite (2:1 v/v) and receiving weekly applications of 50 mL of nutrient solution. During the experiment, humidity and temperature were controlled, and plant toxicity symptoms were monitored as described by Norris and Date (1976).

The cowpea plants were inoculated with a bacterial suspension prepared from cultures in the exponential growth phase in Yeast-Mannitol liquid medium, incubated at 28 °C for three days under constant agitation (Vincent, 1970; Dilworth et al., 2008). A 1 mL aliquot of this suspension was applied to each germinated plant, which had an average height of 10 cm.

Control treatments included: one treatment equivalent to 40 kg of nitrogen per hectare, one without nitrogen application, and one with the recommended inoculant for the crop (SEMIA 6462), prepared similarly to the isolated bacteria. After 45 days of sowing, shoot dry mass production, nodulation capacity (nodule dry mass), and leaf nitrogen content were evaluated.

The experimental data from the selection trials were subjected to analysis of variance, followed by Scott-Knott clustering (Scott and Knott, 1974). Residual normality and homoscedasticity were assessed using the Shapiro-Wilk (Shapiro and Wilk, 1965) and Brown-Forsythe (Brown and Forsythe, 1974) tests, respectively.

For DNA extraction, fresh bacterial colonies were propagated in Yeast-Mannitol Broth (YM). Bacterial pellets were subjected to genomic DNA extraction using the Genomic DNA Purification Kit (Thermo Fisher Scientific), obtaining 100 µL of genomic DNA stored at -20 °C. DNA quantification was performed using a NanoDrop spectrophotometer, and its integrity was checked in 0.8% agarose gel using a 1 kb molecular marker.

The PCR reaction was prepared in a final volume of 25 µL, containing ultrapure water (16.3 µL), enzyme buffer (2.5 µL), dNTPs (1.0 µL), Taq DNA Polymerase (0.2 µL), extracted genomic DNA (3.0 µL), forward primer (1.0 µL), and reverse primer (1.0 µL). The annealing temperature was adjusted to 59 °C, resulting in amplicons of approximately 800 bp for 16S rDNA. The PCR reaction was performed in a thermocycler (Applied Biosystems) with 35 cycles, with temperature adjustments for each primer. PCR products were analyzed by 1.5% agarose gel electrophoresis,

visualized on a transilluminator, and photo-documented under UV light.

After PCR, the 16S rDNA gene product was purified using Exo-SAP, followed by incubation at 37°C for 15 minutes and enzymatic inactivation at 80 °C for 15 minutes. The sequencing reaction (final volume of 10 µL) was prepared with Big Dye buffer (1.5 µL), Big Dye Terminator (0.5 µL), forward and reverse primers (0.7 µL each), and 7 µL of the PCR product. Sequencing was performed on a genetic analyzer (Applied Biosystems 3500) under the following cycling conditions: 96 °C for 1 minute, followed by 35 cycles at 96 °C for 15 seconds, 50 °C for 15 seconds, and 60 °C for 4 minutes.

The obtained sequences were manually aligned based on the electropherogram, compared with reference sequences from BLAST, and deposited in GenBank.

3. Results and Discussion

Shoot dry mass (SDM) ranged from 736.67 to 2.220 mg per plant (Table 2). The nitrogen treatment showed the highest SDM. Treatments AM200, AM76, AM212, AM214, AM210, AM142, AM181, AM136, AM05, AM77, and AM190 exhibited SDM values comparable to SEMIA 6462 and superior to other treatments and the nitrogen-free control.

The nitrogen content in the shoot dry mass (SDMN) ranged from 17.09 to 66.85 mg per plant. SDMN was highest in the nitrogen treatment compared to the others. Treatments AM200, AM76, AM212, AM214, AM210, and AM181 showed SDMN values similar to SEMIA 6462 and higher than other treatments and the nitrogen-free control.

Table 2. Shoot dry mass (SDM), shoot nitrogen accumulation (SDMN), and nodule dry mass (NDM) of cowpea plants inoculated with native isolates

Treatment	SDM	SDMN	NDM
	----------mg plant^{-1}----------		
TESTCN	2220.00 a	66.85 a	-
SEMIA6462	1592.50 b	40.79 b	115.00 a
AM200	1683.33 b	43.88 b	187.50 a
AM76	1457.50 b	34.85 b	160.00 a
AM212	1432.50 b	38.66 b	107.50 a
AM214	1427.50 b	37.91 b	97.50 a
AM210	1427.50 b	35.75 b	122.50 a
AM142	1395.00 b	29.34 c	76.67 a
AM181	1367.50 b	35.31 b	120.00 a
AM136	1360.00 b	27.87 c	100.00 a
AM05	1345.00 b	27.80 c	-
AM77	1270.00 b	25.99 c	110.00 a
AM190	1247.50 b	31.82 c	100.00 a
AM215	1150.00 c	26.99 c	110.00 a
AM195	1137.50 c	26.35 c	147.50 a
AM74	1150.00 c	25.42 c	120.00 a
AM186	1072.50 c	25.36 c	140.00 a
AM56	1072.50 c	22.77 c	106.67 a
AM211	960.00 c	23.92 c	100.00 a
AM143	910.00 c	22.51 c	-
AM189	877.50 c	18.20 c	-
AM73	842.50 c	18.20 c	100.00 a
AM139	796.67 c	17.09 c	90.00 a
AM205	736.67 c	20.46 c	110.00 a
TESTSN	1137.50 c	25.00 c	-

* Means followed by the same letter in the columns do not differ according to the Scott-Knott test ($p < 0.05$).

Nodulation did not differ significantly among inoculated treatments, although treatments AM05, AM143, and AM189 did not induce nodulation. SDM results indicate a positive response to inoculation, with the best performance observed under nitrogen fertilization equivalent to 40 kg N ha^{-1}. According to Costa et al. (2013), no strain (including commercial cowpea inoculants) matched the performance of high mineral nitrogen input, corroborating our findings. Conversely, Melo and Zilli (2009) reported that three out of five cowpea cultivars tested with inoculants in greenhouse conditions did not differ from nitrogen fertilization, and two exceeded it in SDM.

Compared to other studies, isolates AM200, AM76, AM212, AM214, AM210, AM142, AM181, AM136, AM05, AM77, and AM190 performed similarly to SEMIA 6462, within a promising SDM range. In a greenhouse study in Kenya, Nyaga and Njeru (2020) reported SDM values ranging from 840 to 1,050 mg

with native isolates, lower than those observed in this study.

Athul et al. (2022) reported that native rhizobial strains perform better under favorable growth and proliferation conditions, thereby enhancing soil fertility and legume productivity. Although this study was conducted only in a greenhouse, the results support the need for future field trials, especially given the performance equivalence with the recommended strain.

Variation in SDMN values (17.09-66.85 mg/plant) reflects the isolates' efficiency in atmospheric nitrogen fixation. Fernandes et al. (2003) reported similar results, with SDMN ranging from 5.78 to 74.84 mg/plant. The nitrogen fertilization treatment had the highest value, corroborating Costa et al. (2013) and Chagas Junior et al. (2010), though differing from Melo e Zilli (2009), who observed inoculants outperforming fertilization. Even so, AM200, AM76, AM212, AM214, AM210, and AM181 had SDMN values equivalent to SEMIA 6462, confirming their nitrogen-supplying potential.

Regarding nodulation, nodule dry mass (NDM) did not differ significantly among inoculated treatments, except for AM05, AM143, and AM189, which showed no nodulation. This could be related to strain-host compatibility and may depend on temperature. In temperate regions, rhizobial survival and symbiosis can be affected. The optimal growth temperature for rhizobia ranges from 28 to 31 °C. Some strains cannot grow at 38 °C, while others surviving thermal stress may lose nodulation ability due to alterations in infective compounds, such as plasmid modification or changes in cell polysaccharides (Zahran, 1999).

The performance observed in SDM and SDMN may be related to the origin of the isolates. Isolates from Amazonian Dark Earth (ADE) and floodplain soils (FP), such as AM200, AM76, AM212, AM214, AM210, and AM181, showed consistent results, likely due to higher soil fertility. According to Barros et al. (2019), native rhizobial diversity is influenced by soil type, vegetation, and climate variables such as temperature and precipitation.

Molecular analysis based on the 16S rDNA gene revealed greater genetic diversity. While *Rhizobium* was predominant, species from the genera Microbacterium and *Paenibacillus* were also identified (Table 3), consistent with studies reporting microbial diversity in nodules under varied environmental conditions. This highlights the complexity of microbial communities involved in BNF and the importance of integrated approaches to understand isolate efficacy.

The functional characterization of the isolates revealed additional plant growth-promoting traits beyond biological nitrogen fixation, particularly indole-3-acetic acid (IAA) production and calcium phosphate solubilization. These mechanisms are widely recognized as key drivers of plant growth promotion, as microbial IAA stimulates root elongation and branching, thereby enhancing nutrient and water uptake (Spaepen et al., 2007; Patten and Glick, 2002).

Phosphate-solubilizing bacteria contribute to plant nutrition by releasing organic acids that mobilize insoluble phosphorus forms in soil, increasing nutrient availability and improving plant yield (Rodríguez and Fraga, 1999; Sharma et al., 2013). The occurrence of these traits across several isolates in the present study reinforces their multifunctional potential and suggests that their contribution to plant growth may extend beyond nitrogen supply through complementary mechanisms that act on nutrient acquisition and root system modulation (Lugtenberg and Kamilova, 2009; Souza et al., 2015).

Some *Microbacterium* and *Paenibacillus* species identified in this study are known as nodule endophytes, although not traditionally associated with effective nodulation. Kan et al. (2007) suggest these bacteria may co-infect tissues with nodulating strains. Their presence indicates potentially complex interactions during nodulation.

Although not classical rhizobia, *Microbacterium* and *Paenibacillus* have been reported to nodulate cowpea (Marra et al., 2012; Jaramillo et al., 2013). This suggests these endophytes may contribute under certain conditions, possibly through mechanisms not yet fully understood.

Additionally, some studies indicate endophytic bacteria may evolve into symbionts via horizontal gene transfer of symbiotic genes (Li et al., 2008; Shiraishi et al., 2010). This capacity may expand the range of microorganisms with potential applications in BNF and sustainable agriculture.

Molecular techniques are essential for accurate taxonomic identification and should complement phenotypic analyses. Joglekar et al. (2020) highlight the importance of integrating morphological and genetic data for selecting promising strains. This approach enhances the identification of efficient BNF strains.

The diversity revealed by 16S rRNA analysis indicates that cowpea nodules harbor complex microbial communities composed not only of classical rhizobia but also of other plant-associated bacteria. This supports the concept that nodules represent ecological niches where multiple microorganisms coexist and potentially interact functionally (Compant et al., 2010).

From an evolutionary perspective, the presence of genera such as *Microbacterium* and *Paenibacillus* may reflect adaptive processes driven by environmental heterogeneity, including horizontal gene transfer and ecological selection, which can expand symbiotic capabilities within microbial communities (Masson-Boivin et al., 2009; Peix et al., 2015). This functional

diversity indicates the importance of considering microbial consortia when selecting native isolates, as multifunctional communities may provide greater stability under field conditions.

The evaluated isolates, especially AM200, AM76, AM212, AM214, AM210, and AM181, demonstrated BNF potential and agronomic efficiency comparable to SEMIA 6462. Their origin from Amazonian Dark Earth and floodplain soils suggests adaptation to specific edaphic conditions, making them strong candidates for inoculant development in the Amazon.

Table 3. Taxonomic identification and plant growth-promoting traits of the strains used in the study

Species	Isolate	Identity (%)	GenBank accession number	Origin soil	Phosphate solubilization	Indole-3-acetic acid production
Paenibacillus polymyxa	AM05/2022/CPAA	99.24	PQ201611	OX – NE	-	-
Rhizobium tropici	AM56/2022/CPAA	96.89	PQ201612	ADE-NE	+	-
Microbacterium sp	AM73/2022/CPAA	95.96	PQ201613	ADE-NE	-	+
Microbacterium neimengense	AM74/2022/CPAA	97.51	PQ201614	ADE-NE	-	+
Microbacterium neimengense	AM76/2022/CPAA	96.81	PQ424030	ADE-NE	-	-
Microbacterium neimengense	AM77/2022/CPAA	97.84	PQ201615	ADE-NE	-	-
Rhizobium oryzihabitans	AM136/2022/CPAA	97.10	PQ201616	ADE-TU	-	+
Rhizobium oryzihabitans	AM139/2022/CPAA	96.95	PQ201617	ADE-TU	-	+
Rhizobium freirei	AM142/2022/CPAA	96.40	PQ201618	ADE-TU	-	+
Rhizobium sp	AM143/2022/CPAA	96.88	PQ201619	ADE-TU	-	+
Rhizobium sp	AM181/2022/CPAA	79.54	PQ201620	FP-TU	+	+
Rhizobium sp	AM186/2022/CPAA	96.43	PQ201621	FP-NE	-	+
Rhizobium sp	AM189/2022/CPAA	96.38	PQ201622	FP-NE	+	+
Rhizobium sp	AM190/2022/CPAA	96.48	PQ201623	FP-NE	+	+
Rhizobium sp	AM195/2022/CPAA	96.06	PQ201624	FP-TU	+	+
Rhizobium sp	AM200/2022/CPAA	96.49	PQ201625	FP-TU	+	+
Rhizobium sp	AM205/2022/CPAA	97.21	PQ201626	FP-NE	-	+
Rhizobium sp	AM210/2022/CPAA	99.54	PQ201627	FP-NE	-	+
Rhizobium sp	AM211/2022/CPAA	91.05	PQ201628	FP-NE	-	+
Rhizobium sp	AM212/2022/CPAA	96.22	PQ201629	FP-NE	-	+
Rhizobium sp	AM214/2022/CPAA	95.77	PQ201630	FP-NE	+	+
Rhizobium sp	AM215/2022/CPAA	93.92	PQ201631	FP-NE	+	+

FP: Floodplain Soil; OX: Oxisol; ADE: Amazonian Dark Earth; NE: Nova Era; TU: Tumucumaque

4. Conclusions

The results show that several native isolates, particularly AM200, AM76, AM212, AM214, AM210, and AM181, performed comparably to the commercial strain SEMIA 6462 in terms of biomass production, nitrogen accumulation, and nodulation efficiency. Their origin from fertile soils such as Amazonian Dark Earth and floodplain areas may have contributed to this performance.

The observed genetic diversity, combined with agronomic efficiency, reinforces the potential of these isolates for the development of bioinoculants tailored to the Amazon's edaphoclimatic conditions, pending confirmation through field trials. The use of these microorganisms may support sustainable agriculture by reducing dependence on nitrogen fertilizers and enhancing cowpea yield in the region.

Authors' Contribution

Cláudia Majolo conceived and designed the study and wrote the manuscript. July Anne Amaral de Abreu, Natasha Helena Souza Ribeiro, Samara Ferreira Santos, and Elen Lira da Silva performed laboratory and greenhouse experiments. Rogério Perin conducted statistical analyses. Vanessa Ribeiro Reis processed molecular data and prepared figures and tables. Jéssica Pinheiro dos Santos contributed to microbiological review. Marco Antônio Nogueira provided technical support on biological nitrogen fixation. Aleksander Westphal Muniz coordinated the project and supervised all stages. All authors reviewed and approved the final manuscript.

Acknowledgments

The authors acknowledge the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for financial support and scholarships, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research funding and support for scientific and technological innovation in the Amazon.

Bibliographic References

Almeida Neta, M.N., Pegoraro, R.F., Sampaio, R.A., Costa, C.A., Fernandes, L.A., Ferreira, J.M. 2020. Does inoculation with *Rhizobium tropici* and nitrogen fertilization increase chickpea production?. Ciência e Agrotecnologia, 44, e01652. DOI: https://doi.org/10.1590/1413-7054202044016520

Athul, P.P., Patra, R.K., Sethi, D., Panda, N., Mukhi, S.K., Padhan, K., Sahoo, S.K., Sahoo, T.R., Mangaraj, S., Pradhan, S. R., Pattanayak, S. K. 2022. Efficient native strains of rhizobia improved nodulation and productivity of French bean (*Phaseolus vulgaris* L.) under rainfed condition. Frontiers in Plant Science. 13, 1048696, DOI: https://doi.org/10.3389/fpls.2022.1048696

Barros, V.D.C., Lira Junior, M.A., Santos, M.V.F., Costa, A.L., Arruda, A.M., Sousa, C.A. 2019. Biodiversidade rizobiana em função de solo e clima no semiárido pernambucano. Pesquisa Agropecuária Pernambucana, 24(1), e-1951242019. DOI: https://doi.org/10.12661/pap.2019.002

Bertini, C.H.C.M., Teófilo, E.M., Dias, F.T.C. 2008. Divergência genética entre acessos de feijão caupi do banco de germoplasma da UFC. Revista Ciência Agronômica, 40(01), 99-105. ISSN: 1806-6690

Brown, M.B., Forsythe, A.B. 1974. Robust Tests for the equality of variances. Journal of the American Statistical Association, 69, 364-367. DOI: https://doi.org/10.1080/01621459.1974.10482955

Chagas Junior, A.F., Oliveira, L.A., Oliveira, A.N. 2010. Caracterização fenotípica de rizóbio nativos isolados de solos da Amazônia e eficiência simbiótica em feijão caupi. Acta Scientiarum Agronomy, 32(1), 161–169. DOI: https://doi.org/10.4025/actasciagron.v32i1.900

Compant, S., Clément, C., Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants. Soil Biology and Biochemistry, 42, 669–678. DOI: https://doi.org/10.1016/j.soilbio.2009.11.024

CONAB. COMPANHIA NACIONAL DE ABASTECIMENTO. 2024. Ministério da Agricultura, Pecuária e Abastecimento. Acompanhamento da safra brasileira: Grãos. https://www.conab.gov.br (Accessed on May 15, 2024).

Costa, E.M., Nóbrega, R.S.A., Carvalho, F., Trochmann, A., Ferreira, L.V.M., Moreira, F.M.S. 2013. Promoção do crescimento vegetal e diversidade genética de bactérias isoladas de nódulos de feijão-caupi. Pesquisa Agropecuária Brasileira, 48(9), 1275–1284. DOI: https://doi.org/10.1590/S0100-204X2013000900012

Dilworth, M.J., James, E.K., Sprent, J.I., Newton, W.E. 2008. Nitrogen-fixing Leguminous Symbioses, Springer Science, A.A. Dordrecht, The Netherlands. DOI: https://doi.org/10.1017/S0014479708007370

Fernandes, M.F., Fernandes, R.P.M., Hungria, M. 2003. Seleção de rizóbios nativos para guandu, caupi e feijão-de-porco nos tabuleiros costeiros de Sergipe. Pesquisa Agropecuária Brasileira, 38(7), 835–842. DOI: https://doi.org/10.1590/S0100-204X2003000700007

Filgueiras, G.C., Santos, M.A.S., Homma, A.K.O., Rebello, F.K., Cravo, M.S. 2009. Aspectos socioeconômicos. In: Zilli, J.É., Vilarinho, A.A., Alves, J.M.A (Ed.) A cultura do feijão-caupi na Amazônia brasileira. Embrapa Roraima, Boa vista, p. 23-58. ISBN: 978-85-62701-00-9

Hungria, M., Araújo, R.S. 1994. Manual de métodos empregados em estudos de microbiologia agrícola. EMBRAPA-CNPAF, Documentos 46, Brasília. ISSN: 0101-9716

Hungria, M., Campo, R.J., Mendes, I.C. 2007. A importância do processo de fixação biológica do nitrogênio para a cultura da soja: Componente essencial para a competitividade do

produto brasileiro. Embrapa Soja, Londrina. ISSN: 1516-781X; n.283

Jaramillo, P.M.D., Guimarães, A.Z., Florentino, L.A., Silva, K.B., Nóbrega, R.S.A., Moreira, F.M.S. 2013. Symbiotic nitrogen‑fixing bacterial populations trapped from soils under agroforestry systems in the Western Amazon. Scientia Agricola, 70 (6), 397‑404. DOI: https://doi.org/10.1590/S0103-90162013000600004

Joglekar, P., Mesa, C.P., Richards, V.A., Polson, S.W., Wommack, K.E., Fuhrmann, J.J. 2020. Polyphasic analysis reveals correlation between phenotypic and genotypic analysis in soybean bradyrhizobia (*Bradyrhizobium* spp.). Systematic and Applied Microbiology, 43(3), 126073. DOI: https://doi.org/10.1016/j.syapm.2020.126073

Junk, W.J., Piedade, M.T.F, Wittmann, F., Schöngart, J. 2020. Várzeas Amazônicas: Desafios para um manejo sustentável. Editora INPA, Manaus. ISBN: 978-65-5633-005-1

Kan, F.L., Chen, Z.Y., Wang, E.T., Tian, C.F., Sui, X.H., Chen, W.X. 2007. Characterization of symbiotic and endophytic bacteria isolated from root nodules of herbaceous legumes grown in Qinghai–Tibet plateau and in other zones of China. Archives of Microbiology, 188 (2), 103–115. DOI: https://doi.org/10.1007/s00203-007-0211-3

Li, J.H., Wang, E.T., Chen, W.F., Chen, W.X. 2008. Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biology and Biochemistry, 40, 238–246. DOI: https://doi.org/10.1016/j.soilbio.2007.08.014

Lugtenberg, B., Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology, 63, 541–556. DOI: https://doi.org/10.1146/annurev.micro.62.081307.162918

Marra, L.M., Soares, C.R.F.S., Oliveira, S.M., Ferreira, P.A.A., Soares, B.L., Carvalho, R.F., Lima, J.M., Moreira, F.M.S. 2012. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. Plant and Soil, 357, 289‑307. DOI: https://doi.org/10.1007/s11104-012-1157-z

Masson-Boivin, C., Giraud, E., Perret, X., Batut, J. 2009. Establishing nitrogen-fixing symbiosis with legumes. Trends in Microbiology, 17, 458–466. DOI: https://doi.org/10.1016/j.tim.2009.07.004

Melo, S.R., Zilli, J.É. 2009. Fixação biológica de nitrogênio em cultivares de feijão-caupi recomendadas para o Estado de Roraima. Pesquisa Agropecuária Brasileira, 44 (9), 1177–1183. DOI: https://doi.org/10.1590/S0100-204X2009000900016

Messias, M., Asobia, P.C., Ferreira, E.P.B. 2023. Rhizobia and azospirilla co-inoculation boosts growth and productivity of common bean. International Journal of Agriculture and Biology, 29(1), 65-73. DOI: https://doi.org/10.17957/IJAB/15.2004

Moreira, F.M.S.; Siqueira, J.O. 2006. Microbiologia e bioquímica do solo, segunda edição, UFLA, Lavras. ISBN: 85-87692-33-X

Muniz, A.W.; Majolo, C. 2024. Supplemental of Characterization and selection of native rhizobia from Amazonian soils for *Vigna unguiculata* (L.) Walp. OSF. December 5. DOI: https://doi.org/10.17605/OSF.IO/GXNFS

Nyaga, J.W., Njeru, E.M. 2020. Potential of native rhizobia to improve cowpea growth and production in semiarid regions of Kenya. Frontiers in Agronomy, 214. DOI: https://doi.org/10.3389/fagro.2020.606293

Norris D.O., Date, R.A. 1976. Legume Bacteriology. In: Shaw, N.H., Bryan, W.W. (Eds) Tropical pasture research-principles and methods (Commonwealth Bureau Pastures and Field Crops Bulletin), CABI Publishing, Wallingford, 51, p.134-174. ISBN: 978-0851983585

Oliveira, I.J., Fontes, J.R.A., Dias, M.C., Barreto, J.F. 2019. Recomendações técnicas para o cultivo de feijão-caupi no Estado do Amazonas. Circular Técnica, Embrapa Amazônia Ocidental. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/208255/1/Circ-Tec-71.pdf (Accessed on September 7, 2024)

Patten, C.L., Glick, B.R. 2002. Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology, 68(8), 3795–3801. DOI: https://doi.org/10.1128/AEM.68.8.3795-3801.2002

Peix, A., Ramírez-Bahena, M.H., Velázquez, E. 2015. Bacterial associations with legumes. Critical Reviews in Plant Sciences, 34, 17–42. DOI: https://doi.org/10.1080/07352689.2014.897899

Rodríguez, H., Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances, 17, 319–339. DOI: https://doi.org/10.1016/S0734-9750(99)00014-2

Schellekens, J., Almeida-Santos, T., Macedo, R.S., Buurman, P., Kuyper, T.W., Vidal-Torrado, P. 2017. Molecular composition of several soil organic matter fractions from anthropogenic black soils (Terra Preta de Índio) in Amazonia — A pyrolysis-GC/MS study. Geoderma, 288, 154-165. DOI: https://doi.org/10.1016/j.geoderma.2016.11.001

Scott, A.J.; Knott, M. 1974. A Cluster Analysis Method for Grouping Means in the Analysis of Variance. Biometrics, 30(3), 507-512. DOI: https://doi.org/10.2307/2529204

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus, 2, 587. DOI: https://doi.org/10.1186/2193-1801-2-587

Shapiro, S.S.; Wilk, M.B. 1965. An Analysis of variance test for normality (Complete Samples). Biometrika, 52(3/4), 591-611. DOI: https://doi.org/10.2307/2333709

Shiraishi, A.; Matsushita, N.; Hougetsu, T. 2010. Nodulation in black locust by the *Gammaproteobacteria Pseudomonas* sp. and the *Betaproteobacteria Burkholderia* sp. Systematic and Applied Microbiology, 33, 269‑274. DOI: https://doi.org/10.1016/j.syapm.2010.04.005

Shevkani, K.; Shivani, B.; Dhaka, S.S.; Patil, C. 2025. Cowpeas for sustainable agriculture and nutrition security: an overview of their nutritional quality and agroeconomic

advantages. Discov Food, 5, 109. DOI: https://doi.org/10.1007/s44187-025-00382-x

Silva, E.F.L., Araújo, A.S.F., Santos, V.B., Nunes, L.A.P.L., Carneiro, R.F.V. 2010. Fixação biológica do N_2 em feijão-caupi sob diferentes doses e fontes de fósforo solúvel. Bioscience Journal, 26(3), 394-402. https://seer.ufu.br/index.php/biosciencejournal/article/view/7117

Souza, R., Ambrosini, A., Passaglia, L.M.P. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and Molecular Biology, 38, 401–419. DOI: https://doi.org/10.1590/S1415-475738420150053

Spaepen, S., Vanderleyden, J., Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31(4), 425–448. DOI: https://doi.org/10.1111/j.1574-6976.2007.00072.x

Vincent, J.M. 1970. Manual for the practical study of root nodule bacteria. Blackwell Scientific Publications, (IBP Handbook, 15), Oxford. DOI: https://doi.org/10.1002/jobm.19720120524

Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews, 63(4), 968–989. DOI: https://doi.org/10.1128/mmbr.63.4.968-989.1999