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Genetic diversity and symbiotic efficiency of pigeonpea rhizobia from the Brazilian drylands[1]

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ABSTRACT

The pigeonpea (*Cajanus cajan*) crop has a high potential for sustainable agricultural systems in semiarid regions due to its robustness, multifunctionality, and ability to perform biological nitrogen fixation in symbiosis with rhizobia. This study aimed to isolate and characterize native rhizobia at the molecular and symbiotic levels from pigeonpea root nodules cultivated in soils of the Brazilian drylands. A total of 19 bacterial isolates were obtained, 12 of which amplified the symbiotic *nifH* and *nodC* genes, which were subjected to Box-PCR fingerprinting and 16S rRNA gene sequencing. The isolates were classified into the *Bradyrhizobium*, *Rhizobium*, and *Agrobacterium* genera, with a notable genetic diversity observed even among samples from the same region. In a completely randomized greenhouse experiment under gnotobiotic conditions, 11 out of the 12 isolates exhibited symbiotic efficiency comparable to that of the commercial strain *Bradyrhizobium pachyrhizi* BR 2003 in at least one of the evaluated parameters (shoot dry biomass, number of nodules per plant, and N accumulation). The results indicate the presence of promising native rhizobia for use in inoculants adapted to the conditions of the Brazilian semiarid region, particularly the strains ESA 769, ESA 770, ESA 775, ESA 776, and ESA 777. Additionally, the presence of *Agrobacterium* strains capable of forming functional nodules in pigeonpea suggests the occurrence of horizontal gene transfer of symbiotic genes.

KEYWORDS: *Cajanus cajan*, *Bradyrhizobium*, *Rhizobium*, *Agrobacterium*, biological nitrogen fixation.

RESUMO

Diversidade genética e eficiência simbiótica de rizóbios de guandu da região semiárida do Brasil

A cultura do guandu (*Cajanus cajan*) possui alto potencial para sistemas agrícolas sustentáveis em regiões semiáridas, devido à sua robustez, multifuncionalidade e capacidade de realizar a fixação biológica de nitrogênio em simbiose com rizóbios. Objetivou-se isolar e caracterizar rizóbios nativos, provenientes de nódulos radiculares de guandu cultivado em solos do semiárido brasileiro, tanto a nível molecular quanto simbiótico. Foram obtidos 19 isolados bacterianos, dos quais 12 amplificaram os genes simbióticos *nifH* e *nodC* e foram submetidos à caracterização molecular por Box-PCR e ao sequenciamento do gene 16S rRNA. Os isolados foram classificados nos gêneros *Bradyrhizobium*, *Rhizobium* e *Agrobacterium*, com notável diversidade genética observada mesmo entre amostras de uma mesma região. Em um experimento em casa-de-vegetação totalmente casualizado sob condições gnotobióticas, 11 dos 12 isolados demonstraram eficiência simbiótica comparável à da estirpe comercial *Bradyrhizobium pachyrhizi* BR 2003 em pelo menos um dos parâmetros avaliados (biomassa seca da parte aérea, número de nódulos por planta e acúmulo de N). Os resultados indicam a presença de rizóbios nativos promissores para uso em inoculantes adaptados às condições da região semiárida brasileira, particularmente as estirpes ESA 769, ESA 770, ESA 775, ESA 776 e ESA 777. Adicionalmente, a presença de estirpes de *Agrobacterium* capazes de formar nódulos funcionais em guandu sugere a ocorrência de transferência horizontal de genes simbióticos.

PALAVRAS-CHAVE: *Cajanus cajan*, *Bradyrhizobium*, *Rhizobium*, *Agrobacterium*, fixação biológica de nitrogênio.

INTRODUCTION

Pigeonpea (*Cajanus cajan* L. Millsp.) is a multifunctional tropical legume used mainly as a staple food, as well as green manure and forage. Across the tropical world, over one billion people rely on pigeonpea as their primary source of protein, underscoring the significance of this legume crop

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to smallholders worldwide (Odeny 2007, Abebe 2022). Adapting to low-fertility soils and growing vigorously in marginal lands also indicate the potential of pigeonpea for improving the health of degraded soils (Bicalho et al. 2024). Additionally, it is one of the most drought-tolerant crop legumes, indicating its potential for use in drylands (Odeny 2007, Bakala et al. 2024), and is a low-input crop, whose primary macronutrient requirements, such as nitrogen (N) and phosphorus (P), are met through associations with soil microorganisms (Bopape et al. 2022).

Pigeonpea interacts with multifunctional soil microbes, especially N-fixing bacteria (Khoiri et al. 2025). Reported as a promiscuous legume, able to associate with a wide range of rhizobia strains and species, pigeonpea usually associates with α-rhizobia, especially those belonging to the *Bradyrhizobium* and *Rhizobium* genera (Costa et al. 2014, Rufini et al. 2016, Jain et al. 2020, Bopape et al. 2022). However, *Parabukholderia* (β-rhizobia) was also reported as an active pigeonpea microsymbiont, albeit less frequently than α-rhizobia (Singha et al. 2018, Bopape et al. 2022).

Due to its promiscuity, the species associates with diverse native rhizobia. However, most native strains are not symbiotically efficient; instead, they are highly competitive at the nodulation sites, leading to ineffective nodules with a low nitrogen fixation rate (Martins et al. 2003). In this case, selecting efficient soil rhizobia is necessary, and studies on the isolation and characterization of soil-native rhizobia must be conducted under tropical soil conditions. The selected bacteria, with high nitrogenase activity, competitiveness, and adaptation to local conditions, could enhance pigeonpea growth and nitrogen fixation, resulting in plants better adapted to field conditions (Mapfumo et al. 2000).

Nevertheless, the isolation and characterization of pigeonpea rhizobia in different soils is important for the continuous prospecting of new rhizobia strains that are symbiotically effective and adapted to the local conditions of harsh environments, such as the Brazilian dryland soils, for example. In addition to selecting new symbiotically efficient strains, the bioprospection of rhizobia helps in understanding the diversity of soil microbes, indicating the existence of new and undescribed species (Araújo et al. 2017).

Focusing on the selection of efficient rhizobia and the study of soil biodiversity, this study aimed to isolate, molecularly characterize, and assess the symbiotic properties of native pigeonpea rhizobia from soil samples in the Brazilian drylands.

MATERIAL AND METHODS

Between January and March 2020, soils were sampled for the present study, being collected in four locations (Petrolina - Pernambuco state; Juazeiro - Bahia state; and Simplício Mendes - Piauí state) in Northeast Brazil, under semiarid climate conditions, all cropped with different pigeonpea accessions. A composite sample (5-6 subsamples) of the topsoil from each area (0-0.2 m) was taken in the field, conditioned in plastic bags, and transported to the laboratory, where it was kept in a cold chamber (10-12 ºC) before being used for pot filling. The soil samples were sieved (0.5 mm) and used to fill 500-mL polystyrene pots. The soil fertility was analyzed (Teixeira et al. 2017), and the results are shown in Table 1.

Seeds of BRS Mandarim pigeonpea were surface disinfected with 98 % v v^{-1} ethanol (30 seconds), 2 % v v^{-1} sodium hypochlorite (10 min), and washed 10 times with autoclaved distilled water (Somasegaran & Hoben 1994). This genotype was selected for its high productive performance (Matta et al. 2024) and responsiveness to inoculation with selected rhizobial strains (Guimarães et al. 2016).

Pots were irrigated daily with distilled water, and the experiment was conducted over 60 days. At the end of the experiment, the plants were collected, and the nodules detached for bacterial isolation. Nodules were surface-disinfected as described for

Table 1. Chemical characteristics of the soils used in the trap host experiment with pigeonpea (*Cajanus cajan*) BRS Mandarim.

Location	EC	pH	C	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al	H+Al	SB	CEC	V
	mS cm^{-1}		g kg^{-1}	mg dm^{-3}	$cmol_c$ dm^{-3}								%
Simplício Mendes	3.19	5.3	15.4	3.73	0.18	0.45	1.4	1.2	0.1	2.9	3.2	6.1	52.9
Juazeiro	0.96	6.7	8.5	10.57	0.21	0.62	8.8	3.2	0.0	1.7	12.8	14.5	88.4
Petrolina	1.06	6.1	5.0	9.71	0.12	0.23	0.9	0.4	0.0	1.0	1.7	2.6	63.2

the seeds, but with a reduced sodium hypochlorite treatment time of 5 min (Somasegaran & Hoben 1994). Afterwards, the nodules were crushed in Petri dishes with YMA medium (Vincent 1970) and incubated at room temperature (25 ± 1º C) for bacterial growth. Typical rhizobia colonies were streaked on a new plate in succession until a complete purification was achieved. The bacterial strains were stored in 25 % v v^{-1} glycerol at -80 ºC.

All bacteria were grown in YM liquid medium, and the DNA was extracted using the Brasilica® DNA extraction kit (LGC Biotecnologia, São Paulo, Brazil), following the manufacturer's instructions. The DNA was preserved in a freezer at -20 ºC, for the molecular analysis. The bacteria were molecularly authenticated by *nifH* and *nodC* duplex PCR (Silva et al. 2019). PCR products were subjected to horizontal electrophoresis in 1 % (w v^{-1}) agarose gel with Diamond™ Nucleic Acid Dye (Promega, Fitchburg, USA), and the clear bands of both amplicons indicated the presence of both genes. All *nifH*- and *nodC*-positive bacteria were selected for the downstream analysis.

The genetic variability of the culture collection was assessed using Box-PCR fingerprinting, aiming to verify the presence of clonal strains within the collection. The bacterial fingerprinting was assessed by PCR using the primer BOX A1R (CTACGGCAAGGCGACGCTGACG) (Versalovic et al. 1994). PCR products were subjected to horizontal electrophoresis using 1.25 % (w v^{-1}) agarose gel at 90 V for 3 hours. All the BOX-PCR profiles were clustered in a similarity dendrogram using the BioNumerics 7.6 software (Applied Maths, Belgium), with the UPGMA method and the Dice coefficient applied.

Twelve *nifH*- and *nodC*-positive and non-clonal strains were identified by 16S rRNA sequence analysis. PCR amplifications were conducted with 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) universal primers (Lane 1991). The PCR product was visualized in a 1 % (w v^{-1}) agarose gel (120 V for 30 min) and purified using the EasyPure PCR Purification Kit (TransGen Biotech, China). The sequencing reaction was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific, USA), and the product was purified with 3M Sodium Acetate. It was then precipitated in 70 % (v/v) ethanol and resuspended in Hi-Di Formamide. Gel electrophoresis was conducted using a SeqStudio genetic analyzer (Applied Biosystems, USA). The sequences were analyzed with the Sequence Scanner 2.0 software, and high-quality sequences (QV > 20) were selected for comparison analysis against the type strains available in the GenBank database using the BLASTn tool. The sequences were deposited in GenBank under the accession numbers PV628258 to PV628269.

The sequences of our bacterial strains and related strains were used to construct phylogenetic trees. All phylogenetic manipulations were conducted in R 4.1.1 (R Core Team 2023). First, the sequences were aligned using the CrustalW algorithm, within the "DECIPHER" R package (Wright 2016). The distance matrix with the Jukes-Cantor model was constructed with the "ape" R package (Paradis & Schliep 2019), and the phylogenetic trees were plotted with the "ggtree" R package (Yu et al. 2017).

A greenhouse experiment under gnotobiotic conditions was conducted to evaluate the symbiotic ability of the bacterial strains. The used substrate was sterile sand, autoclaved twice, with a minimum of a two-day interval. The inoculants were prepared individually, using the 12 bacteria isolated from pigeonpea in the present study, plus the *Bradyrhizobium pachyrhizi* strain BR 2003, commonly used in commercial pigeonpea inoculants (Brasil 2011). The bacteria were grown in YM liquid medium for six days, and the optical density (OD) was adjusted to 0.5 in a spectrophotometer at 600 nm.

Seeds of the BRS Mandarim pigeonpea were surface disinfected as previously described, and three seeds were sown per pot. At 10 days after germination, the spare plants were removed, and a single plant was left per plot. For the inoculation, an aliquot of 1 mL of each OD-adjusted broth was added to each seed. In addition to the 12 bacteria isolated in the present study and the BR 2003 reference strain, two non-inoculated treatments were also implemented: one with N fertilization and another without, totaling 15 treatments in the greenhouse. The N-fertilized treatment was supplied with ammonium sulfate (0.1 mol L^{-1}) weekly, totaling 100 mg $plant^{-1}$ of N. After the cotyledon fell, the plants were supplied with 50 mL of an N-free nutritive solution weekly (Norris & Mannetje 1964). The experiment was conducted for 60 days, when the plants were collected. The shoots were separated from the roots, and the roots were carefully washed in running tap

water while the nodules were detached and counted. Shoots, roots, and nodules were placed separately in paper bags and oven-dried at 65 ºC, for 5 days, and weighed. The shoots were ground, and the shoot nitrogen content was determined with the semi-micro Kjeldahl method (Liao 1981). The total nitrogen in the shoots was estimated by multiplying the N content (mg g^{-1} $plant^{-1}$) by the shoot dry mass (g $plant^{-1}$).

The experiment employed a completely randomized design, with 15 inoculation treatments and 4 replications. The data analysis was conducted in R 4.1.1 (R Core Team 2023), using the "easyanova" R package (Arnhold 2013). First, the normality of the errors and variance homogeneity were checked using the Shapiro-Wilk and Bartlett tests, respectively. Then, the analysis of variance was performed, followed by the Scott-Knott mean range test ($p < 0.05$).

RESULTS AND DISCUSSION

We retrieved 19 bacterial isolates with typical rhizobial colonies, of which 8 exhibited slow growth (6-7 days) and alkaline pH reaction (characteristic of *Bradyrhizobium*), and 11 were fast growers (2-3 days) with acidic pH reaction (characteristic of *Rhizobium* and *Sinorhizobium*). The duplex PCR reaction for identifying one nodulation and a nitrogenase gene revealed that, among the fast-growing bacteria, seven out of 11 strains showed no amplification for both genes. In contrast, all eight slow-growing strains exhibited the presence of both *nifH* and *nodC*. These results are quite common when assessing rhizobial diversity, particularly in culture collections with fast-growing bacteria, as nodules are often co-infected with non-rhizobial strains (Rodrigues et al. 2021, Silva et al. 2023). Some of these non-rhizobial strains may even have a positive effect on plant-rhizobial interactions (Silva et al. 2023). Bacterial strains belonging to non-rhizobial genera, such as *Klebsiella* and *Bacillus*, are typically fast-growing and reduce the medium pH, which can sometimes lead to them being misidentified as *Rhizobium* or *Sinorhizobium* strains (Silva et al. 2019, Silva et al. 2021). For this reason, the molecular selection of these bacterial strains, using PCR amplification of two symbiotic genes, is a useful strategy for quickly identifying potentially non-rhizobial bacteria within the culture collection.

The Box-PCR evaluation revealed that, among the 12 *nifH* and *nodC* bacteria, there were no clonal isolates, as they exhibited different banding profiles (Figure 1). These findings indicate a large diversity within the culture collection isolated from the soils of Juazeiro, Petrolina and Simplício Mendes. Even within a small rhizobial collection, the absence of clonal isolates is a promising result, reinforcing the rhizobial diversity found in the soils of the Brazilian drylands for other crops such as cowpea (*Vigna unguiculata*) (Oliveira et al. 2020, Sena et al. 2020, Oliveira et al. 2025), peanut (*Arachis hypogaea*) (Santos et al. 2017), and lima bean (*Phaseolus lunatus*) (Chibeba et al. 2020, Rodrigues et al. 2021), among others. Regarding pigeonpea, studies in other Brazilian regions, such as the Midwest (Mato Grosso do Sul) (Costa et al. 2014) and Southeast (Minas Gerais and Rio de Janeiro) (Fernandes Júnior et al. 2012, Rufini et al. 2016), have also indicated a large diversity of pigeonpea rhizobia across Brazil.

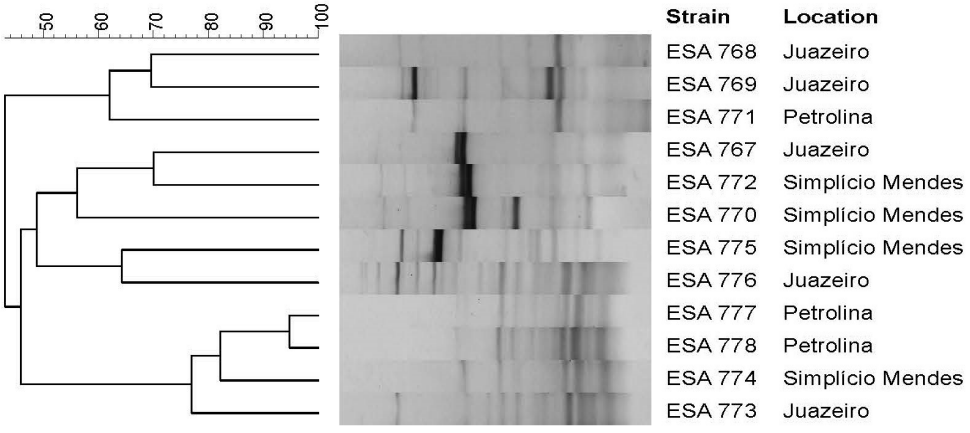

Figure 1. Similarity dendrogram built with the banding patterns of Box-PCR fingerprinting of 12 pigeonpea rhizobial strains. UPGMA methods and the Dice coefficient were used.

The 16S gene sequence analysis showed that the bacterial strains obtained in the present study were classified into two main genetic groups. The strains ESA 767, ESA 768, ESA 769, ESA 771, ESA 772, ESA 776, ESA 777, and ESA 778 were classified within the *Bradyrhizobium* genus (Figure 2A). Among those *Bradyrhizobium* strains, only ESA 771 clustered within the *B. elkanii* superclade and was closely related to *B. embrapense* SEMIA 6208^{T}. The seven other bradyrhizobia were classified within the *B. japonicum* superclade, clustering with *B. ingae* BR 10250^{T} (ESA 772), *B. zhanjiangense* CCBAU 51778^{T} (ESA 778), *B. glycinis* CNPSo 4016^{T} (ESA 767), *B. yuanmingense* NBRC 100594^{T} (ESA 769 and ESA 776), *B. frederickii* CNPSo 3426^{T} (ESA 771), and *B. diversitatis* CNPSo 4019^{T} (ESA 768). The eight *Bradyrhizobium* strains were distributed among the soils sampled in this study.

Pigeonpea is commonly reported as a regular *Bradyrhizobium* macro-symbiont. Additionally, the bacteria within the *B. japonicum* clade are reported to occur at a higher frequency in pigeonpea root nodules than those of *Bradyrhizobium* from other genetic affiliations (Rufini et al. 2016, Bopape et al. 2022). However, the bacterium officially recommended for pigeonpea inoculant production in Brazil is *B. pachyrhizi* BR 2003 (SEMIA 6156), which belongs to the *B. elkanii* supercluster (Zilli et al. 2020), indicating efficient pigeonpea nodulation and N fixation with strains from both main clades. *Bradyrhizobium* strains that are closely related to the same type strains as the pigeonpea rhizobia classified in the present study were already retrieved from soils of the Brazilian drylands using different crops as trap hosts (Oliveira et al. 2020, Sena et al. 2020, Rodrigues et al. 2021, Oliveira et al. 2025), corroborating the large species diversity of bradyrhizobia in soils of the Brazilian drylands.

The bacterial strains ESA 770, ESA 773, ESA 774, and ESA 775 were classified within the *Rhizobium/Agrobacterium* complex (Figure 2B). ESA 770 was related to the *R. qilianshanense* CCNWQLS01^{T}, whereas the bacteria ESA 773 and ESA 775 clustered with *A. salinitolerans* YIC 5082T, and ESA 774 was related to *A. pusense* NRCPB10^{T}. Three out of four *Agrobacterium/Rhizobium* strains were isolated from the Simplício Mendes sampling site, and a single isolate was retrieved from the Juazeiro soil. In addition to *Bradyrhizobium*, pigeonpea is also nodulated by *Rhizobium* and other fast-growing rhizobia (Bopape et al. 2022, Kumar et al. 2023). Despite not being reported as the pigeonpea's

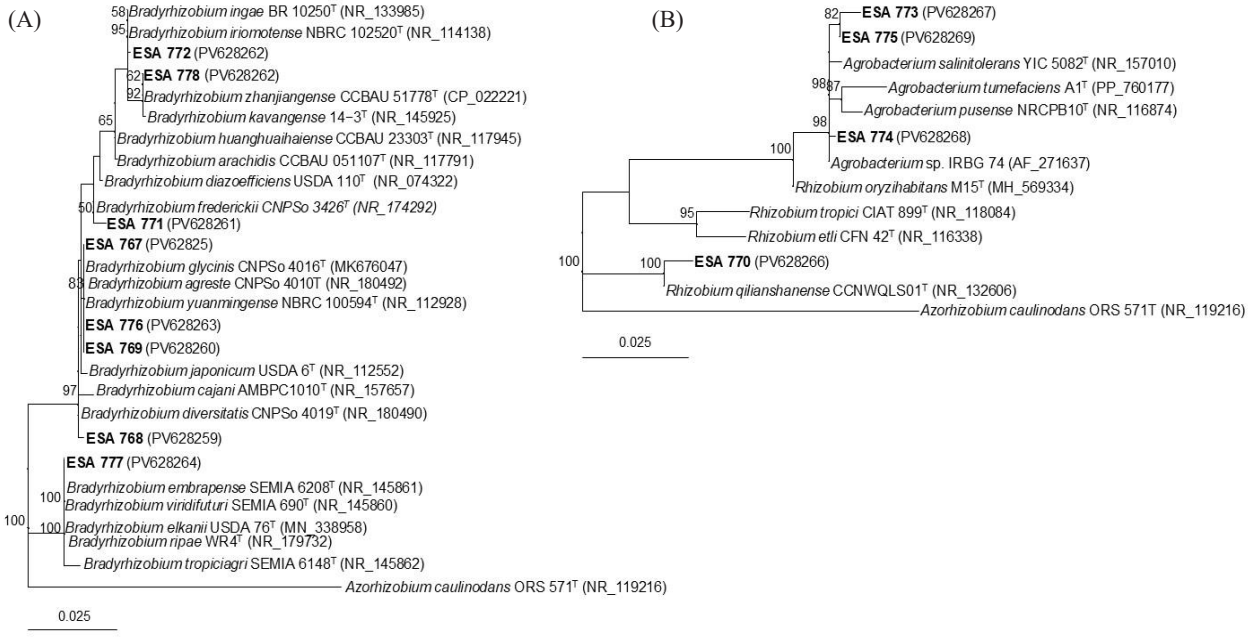

Figure 2. Maximum likelihood 16S rRNA phylogenetic trees of eight pigeonpea strains and 19 *Bradyrhizobium* reference strains (A), and four pigeonpea strains alongside eight *Rhizobium/Agrobacterium* reference strains (B). The final datasets had 1108 and 1255 bp for trees A and B, respectively. The Jukes-Cantor's method and the UPGMA algorithm were used in both trees. *Azorhizobium caulinodans* ORS 571^{T} was used as an outgroup in both trees.

main micro-symbionts, *Rhizobium* and related *Rhizobiaceae* are commonly obtained from pigeonpea root nodules, varying from those classical rhizobia like *Rhizobium*, *Sinorhizobium*, and *Mesorhizobium* (Jain et al. 2020, Bopape et al. 2022) to uncommon *Rhizobiaceae* closely related to *Agrobacterium*, such as *Neorhizobium* (Kumar et al. 2023).

The three bacterial strains classified as *Agrobacterium* in the present study showed more than 99.3 % of similarity with the *Agrobacterium* sp. IRBG74, a well-characterized nodulating *Agrobacterium*. This strain was isolated from root nodules of *Sesbania cannabina* and can re-nodulate the same host alongside other *Sesbania* species (Cummings et al. 2009). Genomic analysis showed that *Agrobacterium* sp. IBPG74 possesses a large (> 500 kb) symbiotic plasmid containing nodulation and nitrogenase genes related to those of *Sinorhizobium meliloti*, suggesting that this bacterium may have acquired this plasmid through horizontal gene transfer (Crook et al. 2013). The *Agrobacterium*-related strains in this study also harbor nodulation genes, as shown by the *nifH* and *nodC* duplex PCR assessment. However, their putative location on symbiotic plasmids still needs further investigation.

The symbiotic effectiveness of the pigeonpea bacterial isolates showed a significant variability in the bacterial efficiency. Eleven bacterial strains exhibited the same shoot dry mass as observed in treatments inoculated with BR 2003 and those supplied with mineral N (Table 2). On the other hand, six inoculation treatments with the pigeonpea strains yielded the same root dry mass as observed in the BR 2003 and N-supplied treatments. The N concentration in the shoots was the same for all inoculated treatments, resulting in 12 inoculation treatments (BR 2003 plus 11 pigeonpea strains) with the same N accumulation in the shoots, which was lower than that observed in the N-fertilized treatment.

Despite several rhizobial species being retrieved from pigeonpea nodules, *Bradyrhizobium* has been reported as the main pigeonpea micro-symbiont (Rufini et al. 2016, Araújo et al. 2017). The symbiotic responses of the inoculated strains in the present study revealed that ESA 773 was the only strain with low symbiotic potential, resulting in lower plant biomass, fewer nodules and reduced N accumulation. This bacterium was classified as an *Agrobacterium* closely related to IBPG74, which, despite being able to nodulate *Sesbania*, also exhibited impaired nodulation with other legumes, such as *Lotus japonicus* (García-Soto et al. 2023). *Bradyrhizobium* from the Brazilian drylands has already been selected for several legumes, including cowpea (Marinho et al. 2017, Sena et al. 2020),

Table 2. Plant dry mass, nodulation, and shoot nitrogen in BRS Mandarin pigeonpea inoculated with 12 rhizobia strains from the Brazilian drylands and *Bradyrhizobium pachyrhizi* BR 2003 under gnotobiotic conditions in a greenhouse experiment.

Inoculation treatment	Plant dry mass			Number of nodules	Shoot nitrogen	
	Shoot	Root	Nodules		Concentration	Accumulation
	g plant^{-1}		mg plant^{-1}	nod plant^{-1}	mg g^{-1}	mg plant^{-1}
ESA 767	1.53 a*	0.76 b	143.75 a	24 a	28.35 b	41.87 b
ESA 768	1.50 a	0.86 b	80.75 a	24 a	26.85 b	40.38 b
ESA 769	1.53 a	1.11 a	135.50 a	30 a	27.76 b	42.50 b
ESA 770	1.68 a	1.02 a	109.75 a	36 a	27.09 b	45.50 b
ESA 771	1.90 a	0.86 b	168.25 a	34 a	30.35 b	59.43 b
ESA 772	1.46 a	0.90 b	115.75 a	28 a	28.53 b	41.57 b
ESA 773	0.98 b	1.19 a	37.25 b	9 b	23.30 b	23.00 c
ESA 774	1.40 a	0.81 b	105.25 a	26 a	26.18 b	34.84 b
ESA 775	2.12 a	0.97 a	160.00 a	42 a	26.64 b	57.46 b
ESA 776	1.81 a	1.17 a	157.50 a	23 a	26.96 b	51.48 b
ESA 777	1.95 a	1.26 a	148.75 a	26 a	25.52 b	49.98 b
ESA 778	1.83 a	0.72 b	114.50 a	19 a	26.84 b	50.30 b
BR 2003	2.02 a	1.03 a	171.00 a	41 a	27.41 b	54.59 b
Nitrogen	2.19 a	1.44 a	0.00 b	0 b	40.68 a	88.49 a
Control	0.50 b	0.49 b	0.00 b	0 b	14.51 c	7.21 d
CV (%)	9.77	29.88	40.03	16.29	15.87	18.24

* Averages followed by the same letter do not differ by the Scott-Knot mean range test (n = 4). CV: coefficient of variation.

soybean (Ribeiro et al. 2015), and peanut (Santos et al. 2017), among others.

The present study contributes another piece to the puzzle of *Bradyrhizobium*-legume association in the Brazilian drylands, indicating the presence of efficient strains within the culture collection. Notably, the study highlights that the strains ESA 769, ESA 770, ESA 776, and ESA 777, in addition to *Agrobacterium* sp. ESA 775, performed well in all variables assessed in the greenhouse experiment. Further investigation is necessary to ensure the efficacy of rhizobia under non-sterile conditions, as well as to dig deeper into the taxonomy and biology of *Rhizobium/Agrobacterium*.

CONCLUSIONS

1. Both *Bradyrhizobium* and *Agrobacterium/Rhizobium* native to the soils of the Brazilian drylands nodulate BRS Mandarim pigeonpea, highlighting the strains ESA 769, ESA 770, ESA 775, ESA 776, and ESA 777, with symbiotic potential for pigeonpea inoculant development;
2. Uncommon rhizobia belonging to *Agrobacterium* harbor symbiotic genes and successfully induce functional nodules in pigeonpea.

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