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Peanut drought tolerance and yield enhanced by new Brazilian *Bradyrhizobium* strains

Abstract – The objective of this work was to identify a new *Bradyrhizobium* strain from *Arachis duranensis* and to evaluate the effects of different bradyrhizobia strains on the performance of two peanut cultivars under conditions of full irrigation and water stress. The ESA 599, ESA 87, ESA 123, and SEMIA 6144 bacterial strains, as well as the BRS 421 OL and BRS 423 OL peanut cultivars, were evaluated. Strain ESA 599 was identified by the 16S rRNA gene sequence analysis and showed less than 96% of similarity to the closest type strain. Plant growth, nodulation, and gas exchanges were evaluated. Strain ESA 599 is phylogenetically distant from the known type strains, suggesting that it is a member of a new lineage from the Brazilian drylands. The ten-day withholding water period impairs the development of both BRS 421 OL and BRS 423 OL cultivars. The inoculation of ESA 123 and ESA 599 enhances plant growth and gas exchanges parameters, especially when there is no water deficit. The inoculation of ESA 123 and ESA 599 benefits differentially the peanut cultivars in the field, indicating a genotypic interaction between macro- and microsymbionts.

Index terms: Biological nitrogen fixation, drought stress, rhizobial inoculants, stress-relieving bacteria.

Tolerância à seca e produtividade de amendoim potencializadas por novas estirpes brasileiras de *Bradyrhizobium*

Resumo – O objetivo deste trabalho foi identificar uma nova estirpe de *Bradyrhizobium* proveniente de *Arachis duranensis* e avaliar os efeitos de diferentes bradyrhizóbios no desempenho de duas cultivares de amendoim sob irrigação plena e estresse hídrico. Foram avaliadas as estirpes ESA 599, ESA 87, ESA 123 e SEMIA 6144, assim como as cultivares BRS 421 OL e BRS 423 OL de amendoim. A estirpe ESA 599 foi identificada por meio da análise da sequência do gene 16S rRNA e apresentou menos de 96% de similaridade com a estirpe-tipo mais próxima. Avaliaram-se o crescimento, a nodulação e as trocas gasosas das plantas. A estirpe ESA 599 é filogeneticamente distante das estirpes-tipo conhecidas, o que sugere que é membro de uma nova linhagem da região semiárida brasileira. O período de dez dias de suspensão de irrigação prejudica o desenvolvimento de ambas as cultivares BRS 421 OL e BRS 423 OL. A inoculação com ESA 123 e ESA 599 melhora o crescimento das plantas e os parâmetros de troca gasosa, especialmente quando não há déficit hídrico. A inoculação com ESA 123 e ESA 599 beneficia as cultivares de amendoim de forma diferenciada no campo, o que indica interação genotípica entre macro- e microsmbiontes.

Termos para indexação: fixação biológica do nitrogênio, estresse hídrico, inoculantes rizobianos, bactérias atenuadoras de estresses.

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Introduction

Peanuts (*Arachis hypogaea* L.) are a valuable crop in Brazil's Northeastern region, esteemed for their adaptability to tropical climates and their crucial role in local agriculture, particularly within family farming systems. However, yields in the region remain low, due to environmental factors, such as water availability and soil fertility (Julião et al. 2022). Official data from CONAB (2025) corroborate this disparity: while the national average peanut yield for the 2024/2025 crop season, harvest in April/May, was approximately 1,700 kg ha⁻¹, the average yield in Northeastern Brazil was only 917 kg ha⁻¹. Therefore, developing environmentally friendly technologies adapted to the region's specific conditions is an urgent priority.

In this context, exploiting the symbiotic relationship between peanuts and soil rhizobia presents a promising approach. While a wide range of soil rhizobia can form symbiotic associations with peanuts, the *Bradyrhizobium* genus, considered the primary microsymbiont for this crop (Santos et al., 2017b). In Brazil, a single strain of *B. elkanii* (SEMIA 6144) has been recommended for commercial peanut inoculant production for over 30 years (Brasil, 2011). However, this strain has shown inconsistent performance across various Brazilian biomes (Santos et al., 2017a; Jovino et al., 2022). Recent studies focusing on the isolation and evaluation of new bacterial strains from Brazilian drylands indicate that the culture collections in Brazil, especially those in the Northeastern region, harbor efficient bradyrhizobial strains with high nitrogen fixation capacity (Sizenando et al., 2016; Jovino et al., 2022). Furthermore, these strains also exhibit complementary mechanisms, such as inducing drought tolerance under conditions of low water availability (Barbosa et al., 2018; Brito et al., 2019).

Beyond microbial approaches, developing genotypes adapted to the Brazilian drylands has become a key strategy for achieving high yields under challenging environmental conditions, such as drought (Santos et al., 2010). Notably, recent releases like the runner cultivars BRS 421 OL and BRS 423 OL demonstrate strong adaptation to the climatic conditions of the Brazilian Northeastern region, making them suitable for both family farming and large-scale production (Suassuna et al., 2020). However, the symbiotic capability of BRS 421 OL and BRS 423 OL peanut cultivars with *Bradyrhizobium* strains has yet to be determined.

Despite the challenging environmental conditions in the Brazilian Northeastern region, especially within the semiarid belt, peanuts and other legumes demonstrate a notable ability to endure drought more effectively (Barbosa et al., 2018; Brito et al., 2019; Correa et al., 2024). Consequently, selecting and assessing novel rhizobial strains can unveil new bacteria offering agronomic benefits beyond nitrogen fixation. This approach enhances plant performance under drought, a crucial characteristic in a changing climate.

The objective of this work was to identify a novel *Bradyrhizobium* strain from *Arachis duranensis* and to evaluate the effects of distinct bradyrhizobia strains on the performance of two peanut cultivars under conditions of full irrigation and water stress.

Materials and Methods

The bacterial strains ESA 599, ESA 87, ESA 123, and SEMIA 6144 were used in the present study. These strains were retrieved from the Culture Collection of Microorganisms with Agricultural Interests (Coleção de Culturas de Microrganismos de Interesse Agrícola) of Embrapa Semiárido, where they are preserved in glycerol stocks at -80°C.

Strain ESA 87, identified as *Bradyrhizobium* sp. by Santos et al. (2017b), was isolated from peanut nodules collected in a soil in the municipality of Juazeiro, in the state of Bahia. ESA 123, identified by Barbosa et al. (2018), is an elite *Bradyrhizobium* sp. strain (Jovino et al., 2022), isolated from peanuts grown in the municipality of Barbalha, in the state of Ceará. In contrast, SEMIA 6144 is a *B. elkanii* strain, officially recommended for commercial peanut inoculant production in Brazil (Brasil, 2011). ESA 599 was isolated from *A. duranensis* and, while its species identification is pending, it has demonstrated the ability to nodulate *A. hypogaeae* (Santos, 2017). The peanut cultivars used in this study were BRS 421 OL and BRS 423 OL, both runner oleic cultivars developed by Embrapa and recognized for their high yield capacity (Suassuna et al., 2020).

For identification of ESA 599, 16S rRNA gene sequencing and analysis were carried out. Bacteria were cultured in liquid Yeast Extract-Manitol (YM) medium for six days at room temperature with continuous stirring at 150 rpm. Bacterial DNA was extracted using the Wizard Genomic DNA Purification

Kit (Promega, Madison, WI, USA). The amplification was conducted on a ProFlex PCR System (Applied Biosystems, Waltham, MA, USA), using the 27F and 1492R universal primers. The resulting PCR products were purified using the EasyPure PCR Purification Kit (TransGen Biotech, Beijing, China). Subsequent sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit, and the products were purified with the BigDye XTerminator Purification Kit (Applied Biosystems, Waltham, MA, USA). Finally, the purified products were sequenced on a SeqStudio Genetic Analyzer (Life Technologies, Waltham, MA, USA).

The 16S rRNA sequences were analyzed using Sequence Scanner 2.0 (Applied Biosystems, Waltham, MA, USA) for quality control. Good-quality sequences (QV > 20) were used for contig assembly and compared against type strains available in the GenBank database using the BLASTn tool. The ESA 599 16S rRNA sequence was deposited in the GenBank database under the accession number PV567554. For phylogenetic analysis, the 16S rRNA sequences of ESA 599, ESA 87 (KY978643), ESA 123 (MG982490), and SEMIA 6144 (AY904750) along with 12 *Bradyrhizobium* type strains and *Bosea thiooxidans* type strain (outgroup) were aligned using the MUSCLE tool within MEGA 11 software (Tamura et al., 2021). Phylogenetic reconstruction was performed using the maximum likelihood method and the Jukes-Cantor model, and was bootstrapped with 1,000 replicates.

The Experiment I was conducted under greenhouse conditions to assess the performance of the four *Bradyrhizobium* strains studied and two non-inoculated controls, with and without nitrogen fertilization. This experiment evaluated the effects of the strains on the growth, gas exchanges, and nodule production of two peanut cultivars subjected to two water availability treatments. The bacteria were retrieved from -80°C glycerol stocks and streaked onto Yeast Extract-Manitol-Agar (YMA) petri dishes. After seven days, the bacteria were transferred to YM broth and cultured under continuous stirring at 150 rpm for 5 days. The bacteria broth was then adjusted to an optical density (OD) of 0.6 in a spectrophotometer at 600 nm ($OD_{600} = 0.6$) in 1X PBS, and the adjusted broth served as the inoculum.

Seeds were surface-disinfected with ethanol 95% (v.v⁻¹) and 2.5% sodium hypochlorite (v.v⁻¹), followed by eight rinses with autoclaved water (Somasegaran & Hoben, 1994). The seeds were sown in 5-L polyethylene pots adapted as lysimeters, following the method developed by Lima et al. (2020). The pots were filled with topsoil layer (0–0.2 m) of Ultisols (Typic Paleudults) (Soil Survey Staff, 2022) collected from a cropland in Campina Grande, PB. The soil was chemically analyzed according to Teixeira et al. (2017), showing the following physico-chemical characteristics: pH in water (1:2.5), 5.7; Ca²⁺, 26.9 mmol_c dm⁻³; Mg²⁺, 18.6 mmol_c dm⁻³; Na⁺, 1.5 mmol_c dm⁻³; K⁺, 3.1 mmol_c dm⁻³; S, 50.1 mmol_c dm⁻³; H+Al, 14.9 mmol_c dm⁻³; CEC (T), 65.0 mmol_c dm⁻³; bases saturation (V), 77.1%; Al³⁺, 0.5 mmol_c dm⁻³; P, 50.4 mg dm⁻³; soil organic matter, 50.4 g kg⁻¹. Thirty days before sowing, lime was applied at a rate of 3.0 Mg ha⁻¹. Fertilization with P and K was conducted using simple superphosphate and potassium chloride, applying 1.6 and 0.4 g pot⁻¹, equivalent to 80 and 20 kg ha⁻¹ of P₂O₅ and K₂O, respectively.

Two seeds were sown per pot, and 1 mL of each adjusted bacteria broth was inoculated onto each seed. The non-inoculated control pots received 1 mL of 1X phosphate-buffered saline (PBS) solution. For the nitrogen fertilized treatment, 2 g of ammonium sulfate (~ 80 kg N ha⁻¹) was added between the seeds (4 to 5 cm depth). Plants were irrigated daily at 8 a.m. to replenish evapotranspirative water losses, which were estimated by daily pot weighing. Soil water content was maintained near field capacity throughout the experiment. Greenhouse conditions were monitored daily, with temperatures ranging from 20°C (minimum) to 35°C (maximum) and an average relative humidity of 67%.

Fifteen days after plant emergence (DAE), thinning was performed to leave one plant per pot. For 30 DAE, pot capacity was maintained at 100%. Following this period, at the R1 phenological stage, irrigation was entirely interrupted for the plants subjected to drought stress, while the fully irrigated treatment remained unchanged. After a 10-day interruption, during which soil water content decreased to approximately 40% of pot capacity, gas exchange analyses were conducted, and the plants were subsequently harvested.

Gas exchanges measurements were conducted between 9:00 and 11:00 a.m. using the infrared gas

analyzer of the LI-6400/XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). Measurements were taken under a constant photosynthetically active radiation (PAR) of $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$, to determine parameters including net photosynthesis (A), leaf transpiration (E), stomatal conductance (gs), and internal CO_2 concentration (Ci). After infrared gas analyses, the plant height was measured from the soil to the top of the main axis with a ruler.

The shoots were severed at the soil level and placed in paper bags. Subsequently, the pot contents were transferred to a 0.5 mm mesh sieve in a sink, where roots were carefully washed under slow-running tap water to remove adhering soil. Nodules were then detached from the roots, counted, and recorded to determine the number of nodules per plant. Shoots, roots, and nodules were placed in separate paper bags, oven-dried at 65°C for five days, and weighed to assess their dry weight.

The Experiment II was conducted under field conditions in the municipality of Campina Grande, in the state of Paraíba, Brazil ($7^\circ 13' 29''\text{S}$ and $35^\circ 54' 18''\text{W}$, at 551 m above sea level), to evaluate the influence of bacterial inoculation on the pod production of the BRS 421 OL and BRS 423 OL cultivars. The inoculation treatments in this experiment included the application of SEMIA 6144, ESA 123, and ESA 599 strains, which were selected based on their superior performance in the first experiment. Additionally, two non-inoculated control treatments, with and without nitrogen fertilization, were included. For inoculant preparation, bacteria broths with $\text{OD}_{600} = 0.6$ were mixed with sterilized peat (pH 7.4) in plastic bags at a ratio of 10 mL of broth per 100 g of peat.

In the nitrogen fertilized treatment, ammonium sulfate was applied at a rate of 50 kg N ha^{-1} , split into two equal applications: 50% at sowing and 50% at 20 days after emergence. The experiment was arranged in a randomized block design with a 2 (peanut cultivars) $\times$ 5 (inoculation treatments) factorial scheme, totaling 40 plots across four blocks.

Soil fertility was assessed as described, and fertilization with K, P, and Ca was conducted according to plant requirements (Cavalcanti, 1998). Plants were sown with a spacing of 0.8 m between rows and 0.2 m within rows, resulting in a useful area of 4.8 m^2 per plot. The experiment was drip-irrigated to meet the

plant requirements throughout the phenological stages, with approximately 600 mm of water applied per cycle.

For seed inoculation, peat-based inoculants were prepared with each strain. Immediately before sowing, seeds were mixed with the inoculants at 25 g of inoculants per 1 kg of seeds and homogenized with 20 mL of a 10% sucrose solution per kg of seed. After hand homogenization, the seeds were dried in the shade for 30 to 40 minutes and then sown. Spontaneous plants were manually removed every 10 to 15 day intervals. At 115 DAE, ten plants per plot were harvested from all treatments. Pods and roots were carefully excavated from the soil, and adhering soil was gently removed from the pods. The pods were then air-dried for 5 days and weighed.

All data were analyzed using version 4.4.1 of R language, with the version 2023.12.1+402 of RStudio interface (R Core Team, 2024). Shapiro-Wilk's and Bartlett's tests were used to assess the normality of errors (residuals) and homoscedasticity, respectively. The number of nodules per plant and nodule dry mass variables were Box-Cox transformed to meet the ANOVA requirements. Three-way and two-way ANOVA were applied for the analysis of Experiments I and II, respectively. The easyanova R package (Arnhold, 2013) was used to perform Bartlett's and Shapiro-Wilk's tests, analysis of variance, and the post-hoc mean comparison test, or Student's t-test. Plots for pod production in Experiment II were generated using the ggplot2 R package (Wickham, 2016).

Results and Discussion

Sequencing of the 16S rRNA gene for strain ESA 599 revealed that its closest type strain was *Bradyrhizobium yuanmingense* NBRC 100594, with a similarity of 95.47% (Figure 1). ESA 599 was also closely related to *Bradyrhizobium* sp. ESA 87, a strain previously isolated from a soil sample in the same region (Santos et al., 2017b). The genus *Bradyrhizobium* is the most commonly associated with commercial peanut cultivars (Santos et al., 2017a, 2017b). The low similarity of ESA 599 to its closest type strain ($< 96\%$) suggests that this bacterium likely belongs to a novel *Bradyrhizobium* lineage. This finding is consistent with rhizobia diversity studies on peanuts in the Brazilian drylands (Santos et al., 2017b) and the Chinese research on *A. duranensis* (Chen et al., 2014).

In the Experiment I, inoculation with SEMIA 6144 increased the height of BRS 423 compared to the control without N or water limitations (Table 1). The 10-day total irrigation interruption reduced all plant peanut height of the BRS 421 OL and BRS 423 OL cultivars in all treatments; however, BRS 423 OL inoculated with ESA 87 maintained a similar plant height regardless of the irrigation strategy. Under full irrigation, ESA 599 resulted in greater shoot biomass in BRS 423 OL cultivar compared to other inoculation treatments and the control without N. Bradyrhizobial inoculation significantly increased nodule dry mass only in BRS 421 OL cultivar under irrigated conditions, when compared to the nitrogen fertilized treatment. For all other conditions, including both irrigated and water-stress treatments for BRS 423 OL cultivar, inoculation had no significant effect on nodule dry mass. Regarding nodule number, inoculation increased nodulation in both cultivars when compared to the non-inoculated and nitrogen fertilized treatments. Overall,

data revealed few significant differences between the two cultivars when subjected to the same irrigation and inoculation treatments.

None of the bradyrhizobial strains assessed in this study were able to improve all plant growth parameters across all experimental conditions. This finding is common in rhizobial selection programs, as demonstrated in studies on cowpea (Marinho et al., 2017), peanuts (Valetti et al., 2016; Santos et al., 2017b), soybeans (Ribeiro et al., 2015), and other legumes. Overcoming this limitation presents a significant challenge that must be addressed in future research.

The leaf gas exchanges analysis revealed a significant impact on the water supply regime on both cultivars (Table 2). The suspension of irrigation reduced stomatal conductance (gs), leaf transpiration (E), and net photosynthesis (A) in both cultivars and across all inoculation treatments. However, some significant differences were observed among the inoculation treatments within each cultivar and irrigation strategy.

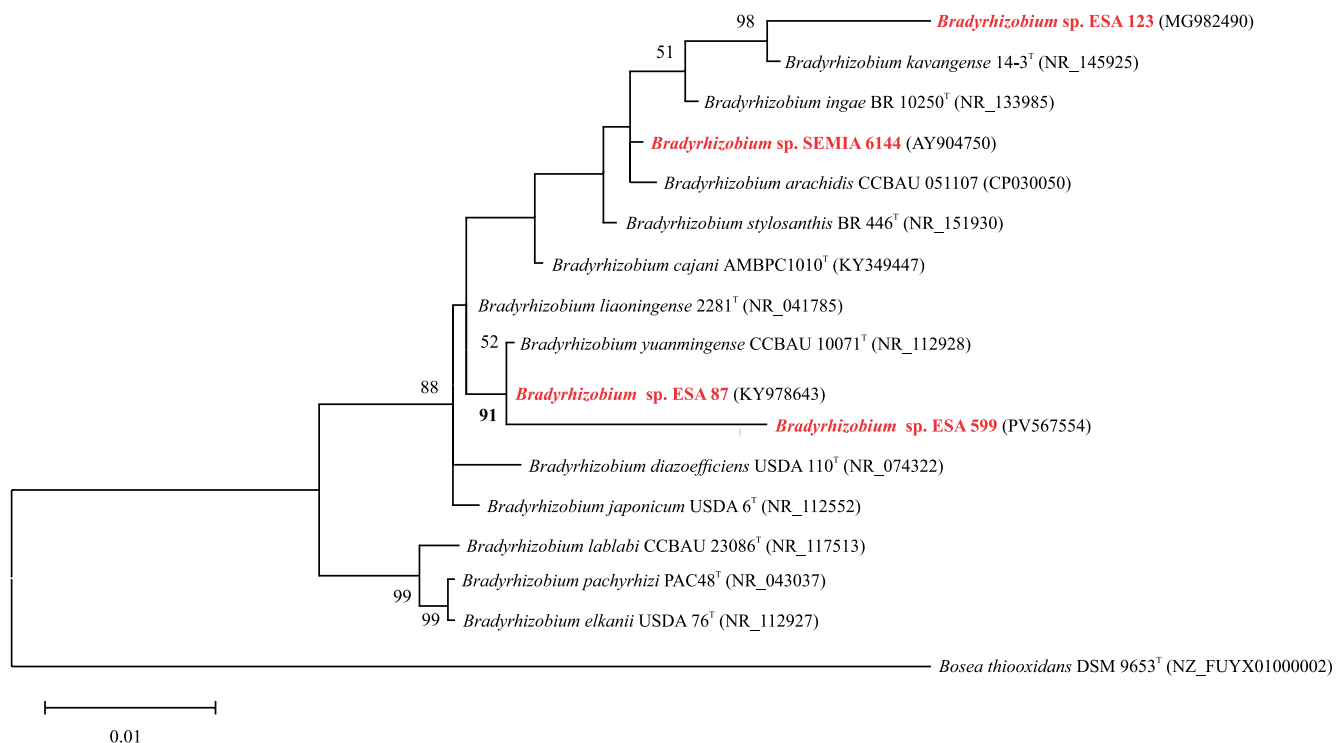


Figure 1. Maximum-likelihood phylogenetic tree of 16S rRNA gene sequences of ESA 87, ESA 123, ESA 599, and SEMIA 6144 (in red) alongside 12 *Bradyrhizobium* type strains (1,260 nucleotides). The Jukes-Cantor model was used for phylogenetic reconstruction; the numbers in the branches represent bootstrap values greater than 50% (1,000 replicates). *Bosea thiooxidans* DSM 9653^T was included as an outgroup. The accession numbers are shown in parentheses. The bar shows the substitution rate.

Table 1. Peanut (*Arachis hypogaea*) growth and nodulation variables of BRS 421 OL and BRS 423 OL cultivars subjected to two different irrigation strategies in a pot experiment under greenhouse conditions⁽¹⁾.

Inoculation	Irrigation strategy			
	Full	Interruption	Full	Interruption
	BRS 421 OL		BRS 423 OL	
Plant height (cm plant ⁻¹), coefficient of variation = 19.44%				
ESA 123	21.4 aAα	16.8 aBα	20.6 abAα	12.0 abcBβ
ESA 599	24.0 aAα	19.0 aBα	21.4 abAα	12.2 abcBβ
ESA 87	24.8 aAα	15.4 aBα	18.0 abAβ	11.2 bcBβ
SEMIA 6144	24.0 aAα	18.2 aBα	17.2 aAβ	10.6 cBβ
Nitrogen	22.0 aAα	17.2 aBα	21.2 abAα	15.8 aBα
Control	20.6 aAα	17.2 aAα	21.0 bAα	15.2 abBα
Shoot dry mass (g plant ⁻¹), coefficient of variation = 24.54%				
ESA 123	7.81 aAα	6.04 aAα	7.28 bAα	5.50 aBα
ESA 599	7.77 aAβ	6.36 aAα	10.81 aAα	5.43 aBα
ESA 87	8.79 aAα	4.97 aBα	9.02 bAα	3.56 aBα
SEMIA 6144	9.27 aAα	6.50 aBα	7.21 bAβ	3.89 aBβ
Nitrogen	8.05 aAα	6.40 aAα	8.98 abAα	4.88 aBα
Control	7.96 aAα	6.11 aBα	7.69 bAα	5.19 aBα
Root dry mass (g plant ⁻¹), coefficient of variation = 29.81%				
ESA 123	0.49 aAα	0.41 aAα	0.47 bAα	0.45 aAα
ESA 599	0.47 aAα	0.43 aAα	0.50 bAα	0.34 aAα
ESA 87	0.49 aAβ	0.38 aAα	0.68 aAα	0.34 aAα
SEMIA 6144	0.61 aAα	0.58 aAα	0.40 bAβ	0.37 aBβ
Nitrogen	0.41 aAα	0.56 aAα	0.44 bAα	0.32 aAβ
Control	0.48 aAα	0.46 aAα	0.53 bAα	0.40 aAα
Nodule dry mass (mg plant ⁻¹), coefficient of variation = 62.00%				
ESA 123	74 abAα	28 aBα	54 aAα	12 aBα
ESA 599	66 bAα	42 aBα	62 aAα	14 aBα
ESA 87	104 abAα	75 aBα	48 aAβ	48 aAα
SEMIA 6144	134 aAα	60 aBα	26 aAβ	18 aAβ
Nitrogen	16 cAα	12 aAα	32 aAα	15 aBα
Control	74 abAα	40 aBα	60 aAα	26 aAα
Number of nodules (nodules plant ⁻¹), coefficient of variation = 54.87%				
ESA 123	24 bcAα	19 aAα	22 abAα	15 abAα
ESA 599	27 abcAα	24 aAα	29 aAα	15 abAα
ESA 87	30 abAα	20 aAα	33 aAα	13 abBα
SEMIA 6144	37 aAα	27 aAα	21 abAα	19 aAα
Nitrogen	9 dAα	12 aAα	16 bAα	6 bAα
Control	17 cdAα	19 aAα	23 abAα	16 abAα

⁽¹⁾Means followed by equal letters, in the same parameter, do not differ from each other by Student's test, at 5% probability. Lowercase letters compare the inoculation treatments within the same irrigation strategy and plant cultivar. Uppercase letters compare irrigation treatments within the same plant cultivar and inoculation treatment. Greek letters compare cultivars within the same inoculation and irrigation treatment.

For example, the stomatal conductance of the BRS 421 OL cultivar was increased by inoculation with ESA 87, ESA 123, and ESA 599, when compared to the non-inoculated control without nitrogen fertilization.

Under the irrigation interruption strategy, no significant differences were observed among the

inoculation treatments or between the two peanut cultivars. The ability to maintain high stomatal conductance suggests the robustness of the plant-rhizobia association, which is a key factor for overcoming low water availability (Barbosa et al., 2018; Brito et al., 2019). The different water regimes

Table 2. Peanut (*Arachis hypogaea*) gas exchange variables for BRS 421 OL and BRS 423 OL cultivars under two irrigation strategies in a pot experiment under greenhouse conditions⁽¹⁾.

Inoculation	Irrigation strategy			
	Full	Interruption	Full	Interruption
	BRS 421 OL		BRS 423 OL	
Stomatal conductance (gs, mmol m ⁻² s ⁻¹), coefficient of variation = 28.70%				
ESA 123	0.26 abAα	0.05 aBα	0.21 cAβ	0.03 aBα
ESA 599	0.26 abAα	0.05 aBα	0.24 bcAα	0.03 aBα
ESA 87	0.26 aAα	0.03 aBα	0.25 abcAα	0.05 aBα
SEMIA 6144	0.22 bcAα	0.06 aBα	0.23 bcAα	0.07 aBα
Nitrogen	0.27 aAα	0.05 aBα	0.29 aAα	0.04 aBα
Control	0.18 cAβ	0.04 aBα	0.27 abAα	0.04 aBα
Leaf transpiration (E, mmol m ⁻² s ⁻¹), coefficient of variation = 22.92%				
ESA 123	2.77 aAα	1.09 aBα	2.69 aAα	0.87 aBα
ESA 599	3.14 aAα	1.26 aBα	2.91 aAα	0.96 aBα
ESA 87	2.94 aAα	0.99 aBα	2.66 aAα	1.22 aBα
SEMIA 6144	2.96 aAα	1.09 aBα	2.64 aAα	1.17 aBα
Nitrogen	2.90 aAα	1.14 aBα	2.81 aAα	1.24 aBα
Control	2.80 aAα	1.17 aBα	3.03 aAα	1.12 aBα
Net photosynthesis (A, μmol CO ₂ m ⁻² s ⁻¹), coefficient of variation = 25.11%				
ESA 123	16.85 aAα	4.49 abBα	15.56 bcAα	3.71 aBα
ESA 599	16.46 aAα	6.50 aBα	20.28 aAα	2.52 aBβ
ESA 87	18.05 aAα	2.49 bBα	17.18 bAα	4.19 aBα
SEMIA 6144	16.47 aAα	3.59 abBα	13.57 cAβ	4.65 aBα
Nitrogen	17.81 aAα	5.34 abBα	21.28 aAα	3.01 aBα
Control	17.25 aAβ	3.81 abBα	15.32 bcA	2.39 aBα
Internal CO ₂ concentration (Ci, μmol CO ₂ mol ⁻¹), coefficient of variation = 15.81%				
ESA 123	252.0 aAα	240.2 bcAα	247.2 aAα	183.6 bBβ
ESA 599	247.4 abAα	233.4 bcAβ	260.0 aAα	279.8 aAα
ESA 87	220.4 abBα	305.0 aAα	193.0 bBα	276.6 aAβ
SEMIA 6144	227.0 abAα	219.8 bcAβ	254.2 aAα	263.4 aAα
Nitrogen	235.8 abAα	197.4 cAβ	218.0 abBα	263.8 aAα
Control	205.0 bBα	245.4 bAβ	245.2 aAα	306.2 aAα

⁽¹⁾Means followed by equal letters, in the rows, do not differ from each other by Student's test, at 5% probability. Lowercase letters compare the inoculation treatments within the same irrigation strategy and plant cultivar. Uppercase letters compare irrigation treatments within the same plant cultivar and inoculation treatment. Greek letters compare cultivars within the same inoculation and irrigation treatment.

across all treatments indicated that withholding water for ten days severely impacted the plant physiology of BRS 421 OL and BRS 423 OL cultivars.

Under full irrigation conditions, inoculation with ESA 599 and nitrogen treatment resulted in higher net photosynthesis rates in BRS 423 OL cultivar compared to SEMIA 6144 strain; however, no significant differences were detected in BRS 421 OL cultivar. Under irrigation restriction, no significant differences in leaf net photosynthesis were observed among treatments. The inoculation of the BRS 423 OL cultivar with ESA 123 under water deficit reduced the internal CO₂ concentration (C_i) inside stomatal chambers, indicating higher efficiency in C incorporation through photosynthesis. The higher photosynthetic activity of legumes in association with rhizobia is likely related to the efficiency of the bacteria isolate in providing N to the plant, as well as other plant-growth-promoting mechanisms (Andrade et al., 2021; Jaiswal & Dakora, 2025).

The Experiment II for assessing peanut yield revealed substantial effects of both the inoculation treatment

and the peanut cultivar (Figure 2). The average yields were 1,865.7 ($\pm$ 297.4) and 1,565.9 ($\pm$ 243.9) kg ha⁻¹ for cultivars BRS 421 OL and BRS 423 OL, respectively, with a statistically significant difference (Student's t test, $p < 0.061$). Within BRS 421 OL, inoculation of ESA 599 resulted in the highest pod production, achieving 2,316.4 ($\pm$ 221.4) kg ha⁻¹, which was substantially higher than the other five treatments, this is, 28.5% higher than the non-inoculated and non-fertilized control. Within BRS 423 OL cultivar, the inoculation of ESA 123 resulted in the highest pod production of 1,801.6 ($\pm$ 269.3) kg ha⁻¹, surpassing the treatments inoculated with SEMIA 6144 and the control without inoculation, while not being statistically different from the ESA 599 inoculation and the nitrogen fertilized treatment. A comparison between cultivars showed that BRS 421 OL plants inoculated with ESA 599 had higher yields than BRS 423 OL plants inoculated with the same bacterium (F test, $p < 0.072$). No substantial differences were found between the two cultivars for the other treatments.

The soils of the Brazilian semiarid region harbor efficient *Bradyrhizobium* strains for peanuts (Santos et al., 2017a, 2017b; Jovino et al., 2022). ESA 123 is an elite *Bradyrhizobium* strain that meets the requirements for official recommendation as a peanut inoculant, having demonstrated high agronomic performance in field trials under various conditions in the Brazilian Northeastern region (Jovino et al., 2022). Furthermore, the novel strain ESA 599, isolated from *A. duranensis*, exhibited improved agronomic performance when associated with BRS 421 OL cultivar.

Conclusions

1. The novel *Bradyrhizobium* strain ESA 599, isolated and evaluated in the present study, is phylogenetically distant from the known type strains, suggesting it as a member of a new lineage from the Brazilian drylands.

2. The 10-day water withholding period impairs the development of both BRS 421 OL and BRS 423 OL cultivars.

3. The inoculation of ESA 123 and ESA 599 enhances plant growth and gas exchanges, especially when there is no water deficit.

4. The inoculation of ESA 123 and ESA 599 differentially benefits the peanut cultivars, indicating

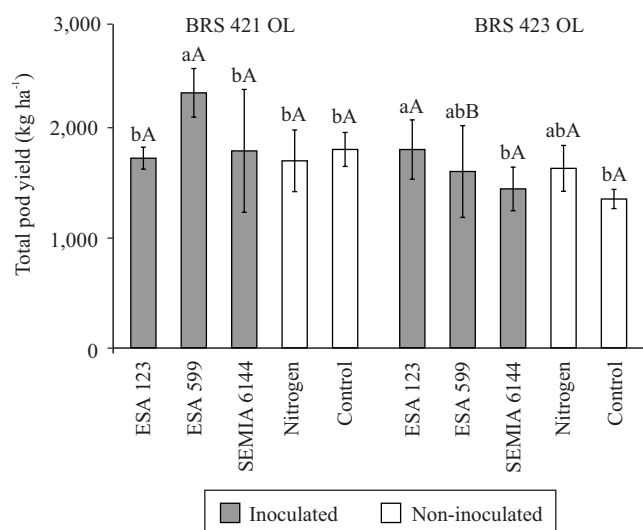


Figure 2. Total pod yield of BRS 421 OL and BRS 423 OL peanut (*Arachis hypogaea*) cultivars inoculated with *Bradyrhizobium* spp. Strains: ESA 123, ESA 599, and SEMIA 6144, or non-inoculated with (Nitrogen) and without (Control) nitrogen fertilization. Bars with the same letter do not differ by Student's test, at 1% probability. Lowercase and uppercase letters compare the inoculation treatments (within the same genotype) and the genotypes (within the same inoculation treatment), respectively. Vertical bars are the error mean deviation.

a genotypic interaction between macro- and microsymbionts.

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José Edson Lourenço dos Santos: data curation, investigation; **Geisenilma Maria Gonçalves da Rocha:** data curation, investigation, supervision; **Laysla Lopes Nunes:** investigation; **Jonnathan Whiny Moraes dos Santos:** investigation, software, writing – review & editing; **Mirandy dos Santos Dias:** formal analysis, investigation, methodology; **Pedro Dantas Fernandes:** data curation, investigation, methodology, resources, supervision; **Tarcísio Marcos de Souza Gondim:** conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, writing – review & editing; **Liziane Maria de Lima:** conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing; **Paulo Ivan Fernandes Júnior:** conceptualization, formal analysis, methodology, project administration, software, supervision, visualization, writing – original draft.

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Data availability statement

The data supporting the findings of this study are available in the article. Should any raw data be needed, they will be provided by the corresponding author upon reasonable request.

Declaration of use of AI technologies

During the preparation of this work, the author(s) used the Grammarly tool in order to double check the language mistakes. After this use, the authors reviewed and edited the content as needed and take full responsibility for it.

Conflict of interest statement

The authors declare no conflicts of interest.

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