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Effect of the biological inoculant Rizokop (Bradyrhizobium japonicum) on soybean emergence and early vigor under different water regimes

Efeito do inoculante biológico Rizokop (Bradyrhizobium japonicum) na emergência e no vigor inicial da soja sob diferentes regimes hídricos

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ABSTRACT

Soybean (Glycine max (L.) Merrill) is one of the main agricultural crops in Brazil, but environmental factors such as water deficit, waterlogging, and high temperatures can compromise emergence, root development, and nodulation. This study aimed to evaluate the effect of the biological inoculant Rizokop, based on Bradyrhizobium japonicum, on the emergence and early vigor of soybean under different water regimes. The pot experiment followed a completely randomized design with six treatments (with and without Rizokop under ideal water supply, water deficit, and water excess) and five replicates, using the cultivar TMG Ingá I2X sown on August 26, 2026. Emergence, emergence speed index (ESI), nodule number and diameter, root length, and root fresh mass were assessed up to the V5 stage, and the data were submitted to analysis of variance and Tukey's test at 5%. Emergence (13.3 to 60.0%) and ESI did not differ among treatments ($p > 0.05$), whereas nodule number ($p < 0.001$), root length ($p < 0.001$), and root fresh mass ($p = 0.022$) did. Inoculated plants had on average 36.3 nodules versus 4.8 in non-inoculated ones and, under water deficit, only inoculated plants nodulated (32.5 versus 0 nodules per plant). The results suggest that Rizokop favored nodulation and early root growth; however, the small number of evaluated plants and the low emergence in some treatments call for caution, and further trials with more replicates are recommended.

Keywords: Abiotic stress; Biological inoculant; Nodulation; Rizokop; Soybean.

RESUMO

A soja (Glycine max (L.) Merrill) é uma das principais culturas agrícolas do Brasil, mas fatores ambientais como déficit hídrico, encharcamento e altas temperaturas podem comprometer a emergência, o desenvolvimento radicular e a nodulação. Este estudo teve como objetivo avaliar o efeito do inoculante biológico Rizokop, à base de Bradyrhizobium japonicum, sobre a emergência e o vigor inicial da soja sob diferentes regimes hídricos. O experimento em vasos seguiu delineamento inteiramente casualizado, com seis tratamentos (com e sem Rizokop sob suprimento hídrico ideal, déficit hídrico e excesso hídrico) e cinco repetições, utilizando a cultivar TMG Ingá I2X, semeada em 26 de agosto de 2026. A

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emergência, o índice de velocidade de emergência (IVE), o número e o diâmetro dos nódulos, o comprimento radicular e a massa fresca de raízes foram avaliados até o estádio V5, e os dados foram submetidos à análise de variância e ao teste de Tukey a 5%. A emergência (13,3 a 60,0%) e o IVE não diferiram entre os tratamentos ($p > 0,05$), ao passo que o número de nódulos ($p < 0,001$), o comprimento radicular ($p < 0,001$) e a massa fresca de raízes ($p = 0,022$) diferiram. As plantas inoculadas apresentaram, em média, 36,3 nódulos, contra 4,8 nas não inoculadas, e, sob déficit hídrico, apenas as plantas inoculadas nodularam (32,5 versus 0 nódulos por planta). Os resultados sugerem que o Rizokop favoreceu a nodulação e o crescimento radicular inicial; contudo, o pequeno número de plantas avaliadas e a baixa emergência em alguns tratamentos exigem cautela, e recomendam-se novos ensaios com mais repetições.

Palavras-chave: Estresse abiótico; Inoculante biológico; Nodulação; Rizokop; Soja.

1 INTRODUCTION

Soybeans (Glycine max (L.) Merrill) have established themselves as one of the world's leading agricultural commodities and occupy a strategic position in the Brazilian agribusiness sector, due to their significant share of exports, their contribution to income generation, and their role in strengthening a production chain that includes vegetable oil, protein meal, human and animal feed, and biodiesel.

Brazil has become the world leader in soybean production and exports. In the 2024/2025 crop year, Brazilian soybean production reached 350.2 million metric tons, setting a new record in the historical series (CONAB, 2025).

This leading role was achieved in just a few decades: domestic production, which was less than 20 million metric tons in the 1980s, exceeded 168.3 million metric tons in the 2024/2025 harvest—a new all-time record (CONAB, 2025)— and agribusiness accounts for approximately 24.4% of the national Gross Domestic Product (CNA, 2025).

In Tocantins, the expansion of soybean cultivation was facilitated by the edaphoclimatic conditions of the Cerrado, such as the relatively flat terrain, the availability of mechanizable areas, and soils with high production potential following chemical correction and proper management. In the 2025 harvest, state soybean production totaled 5,075,812 metric tons, a 15.81% increase compared to the previous harvest (Tocantins, 2026).

However, irregular rainfall, high temperatures, and frequent water deficits have limited the crop's full development, particularly during the early stages of germination and seedling establishment (Vital *et al.*, 2022). These factors constitute abiotic stresses, defined by Taiz *et al.* (2017) as environmental conditions that cause physiological changes capable of

compromising plant metabolism and which, in soybeans, include water deficit, excess soil moisture, and high temperatures.

Studies show that these stresses impair water uptake, photosynthetic activity, osmotic balance, antioxidant metabolism, root growth, nodulation, and final crop yield (Wang *et al.*, 2022; Vital *et al.*, 2022). Water deficit is one of the main factors responsible for reduced soybean productivity (Sinclair; Vadez, 2012), and excess water reduces oxygen diffusion into the rhizosphere, limiting root growth and biological nitrogen fixation (Fageria; Baligar; Clark, 2006).

High temperatures, in turn, alter enzymatic activity and the water balance of plants (Hatfield; Prueger, 2015), reducing germination and impairing root growth (Guan *et al.*, 2022). Furthermore, the increased frequency of extreme weather events has amplified the negative impacts of excessive heat and water scarcity on soybeans, making it necessary to develop sustainable strategies capable of increasing plant tolerance to adverse conditions (Guan *et al.*, 2022).

In this context, biological inoculants have been gaining prominence in agriculture because they promote plant growth, nodulation, and biological nitrogen fixation (Vessey, 2003). In soybean cultivation, studies on the co-inoculation of Bradyrhizobium spp. and Azospirillum brasilense demonstrate the potential to increase productivity and contribute to the sustainability of the production system (Hungria; Nogueira; Araujo, 2015).

In soybeans, biological nitrogen fixation occurs through symbiosis with bacteria of the genus Bradyrhizobium, which form nodules on the roots and supply a large portion of the nitrogen required by the plant, reducing dependence on mineral nitrogen fertilizers (Hungria; Campo; Mendes, 2007; Silva *et al.*, 2024). The efficiency of this symbiosis, however, is sensitive to water deficit, high temperatures, and soil acidity (Hungria; Vargas, 2000).

Research shows that the co-inoculation of plant-growth-promoting bacteria, such as Bradyrhizobium japonicum, Azospirillum brasilense, Pseudomonas putida, and Bacillus species, contributes to increased vigor, root development, nutrient uptake, productivity, and soybean tolerance to water stress (Jabborova *et al.*, 2021; Rocha *et al.*, 2024).

These microorganisms promote the production of phytohormones and stimulate the root system (Vessey, 2003; Bhattacharyya; Jha, 2012)—effects that are particularly relevant during the early stages, when seedling vigor, as reflected by the speed and uniformity of emergence and root development, determines crop establishment (Marcos-Filho, 2015).

Given this context, the present study is justified by the need to expand scientific knowledge on the use of biological inoculants—especially Rizokop—in mitigating the effects

of abiotic stresses on soybean emergence and initial vigor, thereby contributing to the development of sustainable technologies that enable greater yield stability, physiological efficiency, and crop adaptation to the edaphoclimatic conditions of the Cerrado.

Thus, the overall objective of this study was to evaluate the effect of applying the biological inoculant Rizokop on the emergence and initial vigor of soybeans subjected to different water regimes (optimal, deficit, and excess) under the region's natural temperature conditions. Specifically, the study sought to determine emergence and the emergence rate index (ERI), quantify nodulation, and evaluate root growth. Since temperature was not manipulated, thermal stress was considered only as a contextual condition, not as a factor under evaluation.

2 MATERIALS AND METHODS

2.1 RESEARCH LOCATION AND TIME FRAME

The study was conducted at Afya Faculdade Porto Nacional, located in the municipality of Porto Nacional, Tocantins, at the geographic coordinates 10°41′43.7″ S and 48°23′06.7″ W, in a facility designed for conducting experimental agronomic trials in pots, during the 2026/2 season.

Seeding took place on August 26, 2026, and emergence assessments were conducted on August 29, 2026, and August 30, 2026.

The municipality of Porto Nacional is located in the central region of Tocantins, on the banks of the Tocantins River and approximately 60 km from Palmas, the state capital, within the Cerrado biome. It has a land area of 4,434.680 km² and a population of 64,418 inhabitants (IBGE, 2022).

It is a municipality with an agricultural and livestock-based economy, with a focus on livestock and the cultivation of grains, such as soybeans. It also stands out as a hub for services and higher education and for housing a historic urban complex listed by IPHAN in 2008, which makes it representative for agronomic studies under the conditions of the Tocantins Cerrado.

2.2 PLANT MATERIAL AND BIOLOGICAL INOCULANT

The choice of plant material was based on the climatic conditions observed in the region, primarily due to irregular rainfall and the shortened planting window for soybeans.

Thus, the use of cultivars adapted to regional conditions can provide greater assurance in the initial establishment of the crop, reducing potential losses caused by the need for replanting.

The TMG Ingá I2X cultivar was used, which belongs to maturity group 6.9 and was developed using Intacta2 Xtend (I2X) technology. According to information provided by TMG ([2025]), the cultivar exhibits high yield potential, excellent plant architecture, and good tolerance to lodging. In addition, it is resistant to the herbicides glyphosate and dicamba, as well as to cyst nematodes of races 3, 6, and 9, and exhibits moderate resistance to race 10.

The biological treatment was carried out using the commercial inoculant Rizokop, based on Bradyrhizobium japonicum, applied according to the protocol described in section 2.4. According to Koppert Brasil (2026), the product contains the strains SEMIA 5079 and SEMIA 5080 and is recommended for soybean cultivation, contributing to nodulation and the efficiency of biological nitrogen fixation. The official website also states that the product should be applied at the dosage indicated for the specific application scenario.

2.3 EXPERIMENTAL DESIGN

Following the principles of agricultural experimentation, the experiment was conducted using a completely randomized design consisting of six treatments and five replicates, for a total of 30 experimental units. Each unit consisted of a 10-liter pot in which three seeds were sown. After emergence, thinning was performed, leaving only one plant per pot. The treatments were distributed as shown in Table 1.

Table 1 – Description of the experimental treatments

Treatment	Inoculation (Rizokop)	Water availability	Thermal condition
T1 – Control	Without Rizokop	Ideal (5 to 7 mm/day; 157.1 to 219.9 mL/cup/day)	A native of Tocantins
T2 – Rizokop + ideal water	With Rizokop	Ideal (5 to 7 mm/day; 157.1 to 219.9 mL/cup/day)	A native of Tocantins
T3 – Deficit Without Vaccination	Without Rizokop	Water deficit (2 mm/day; 62.8 mL/pot/day)	A native of Tocantins
T4 – Rizokop + deficit	With Rizokop	Water deficit (2 mm/day; 62.8 mL/pot/day)	A native of Tocantins
T5 – Excess without inoculation	Without Rizokop	Excess water (10 mm/day; 314.2 mL/pot/day)	A native of Tocantins

T6 – Rizokop + excess	With Rizokop	Excess water (10 mm/day; 314.2 mL/pot/day)	A native of Tocantins

Source: The authors (2026).

The pots were arranged randomly in order to minimize any potential environmental interference with the results.

2.4 SETUP AND EXPERIMENTAL PROCEDURE

The 10-liter pots were filled with soil collected from the research site itself, from the 0–20 cm layer, mixed with cured cattle manure in a 1:3 ratio (soil:cattle manure, by volume). The material was sieved and homogenized before filling the pots, ensuring uniform conditions for seedling development. The characterization presented below refers to the soil, as the manure was not analyzed.

Cattle manure was incorporated into the soil to improve the physical, chemical, and biological characteristics of the substrate (Silva; Raimundo; Forti, 2019). The soil was used without sterilization or any treatment to eliminate microorganisms, and there was no prior quantification of the native rhizobium population.

The soil was analyzed by HEMA Engenharia (Porto Nacional, TO), in accordance with Embrapa's Manual of Soil Analysis Methods (Teixeira *et al.*, 2017), using samples from the 0–20 cm and 20–40 cm layers.

In the 0–20 cm layer, the soil had a clay texture (520 g/kg of clay; 480 g/kg in the 20–40 cm layer), a $CaCl_2$ pH of 5.08, phosphorus (Mehlich-1) of 0.5 mg/dm³, potassium of 62.79 mg/dm³, Ca and Mg of 1.2 cmolc/dm³ each, H + Al of 3.46 cmolc/dm³, and CTC at pH 7 of 6.22 cmolc/dm³.

According to the criteria for Cerrado soils (Sousa; Lobato, 2004), the phosphorus content was classified as very low and the potassium content as medium, and aluminum saturation was low (2.82%). Based on this analysis, the liming requirement and the phosphate and potassium fertilizer rates were calculated.

The liming requirement (NC) was calculated using the base saturation method, based on data from the 0–20 cm layer, a desired base saturation (V2) of 50%—a value adopted for Cerrado soils (Sousa; Lobato, 2004)—and calcitic limestone with a PRNT of 100%:

$$LC\ (t/ha) = [CTC \times (V2 - V1)] / PRNT$$

The current base saturation (V1) was recalculated based on the sum of bases (Ca + Mg + K = 1.2 + 1.2 + 0.161 = 2.561 cmolc/dm³), resulting in V1 = (2.561 / 6.22) × 100 = 41.17%.

This was necessary because the sum of bases reported in the analysis (2.76 cmolc/dm³, V = 44.38%) does not match the sum of the reported exchangeable cations. Thus:

$$NC = 6.22 \times (50 - 41.17) / 100 = 0.549 \text{ t/ha}$$

Since 1 t/ha in the 0–20 cm layer is equivalent to 0.5 g/dm³ of soil, the NC corresponds to 0.275 g of limestone per dm³ of soil. For phosphorus, the reference rate of 90 kg/ha of P_2O_5 was adopted, consistent with the gradual corrective fertilization for clay soils in the Cerrado with very low P content (Sousa; Lobato, 2004), and for potassium, 60 kg/ha of K_2O. The fertilizers used were triple superphosphate (46% P_2O_5) and potassium chloride (60% K_2O).

Since the growing medium was prepared in a 1:2 ratio (soil:cattle manure, by volume), each 10-L pot contained approximately 3.33 dm³ of soil, and the fertilizer rates were calculated for this volume. The limestone was estimated at 0.275 g/dm³ × 3.33 dm³ = 0.92 g per pot. The triple superphosphate was estimated at (45 mg/dm³ × 3.33 dm³) / 0.46 = 326 mg, or 0.33 g, and potassium chloride at (30 mg/dm³ × 3.33 dm³) / 0.60 = 167 mg, or 0.17 g, totaling 1.42 g of inputs per pot.

The recommendation called for incorporating limestone into the soil, allowing for a reaction period of 20 to 30 days. The application was carried out 21 days before planting (August 5, 2026), followed by the incorporation of triple superphosphate and potassium chloride before sowing (August 26, 2026).

The composted cattle manure was not analyzed, and its nutrient and base contributions were not considered in the calculations; therefore, the application rates represent the requirements of the soil alone.

Seeding was carried out on August 26, 2026, with three seeds per pot. In the treatments with Rizokop (T2, T4, and T6), the seeds were inoculated before sowing; in the other treatments (T1, T3, and T5), the seeds received neither inoculant nor any other product.

Inoculation followed the experimental protocol established based on the Rizokop technical brochure consulted at the time. This document specified, for seed treatment, a reference dose of 100 mL of the product per 50 kg of seeds and, for application in the planting furrow, 300 mL/ha, with a minimum flow rate of 40 L/ha (Koppert Brasil, 2023). It should be noted that the official website currently available does not specify a fixed dose and advises that the dosage be determined according to the crop and specific application conditions, following consultation with a technical representative. Therefore, the values from the technical brochure are recorded here as a historical reference for the protocol, not as a current universal recommendation (Koppert Brasil, 2026).

In areas where soybeans are being grown for the first time, or where the inoculant has never been used, the Bradyrhizobium population in the soil is nonexistent or very low, and Embrapa recommends that the inoculant dose be at least double that indicated for previously cultivated areas (Hungria; Nogueira, 2020).

Since the inoculation history of the soil sampling area was unknown, the experimental dose of 200 mL of Rizokop per 50 kg of seeds was adopted as a precaution, corresponding to twice the reference dose indicated in the technical brochure consulted. The total volume of liquid applied to the seeds was kept below the 300 mL/50 kg limit reported on the official Koppert Brasil website (2026).

Since the trial was conducted in 10-liter pots, with three seeds per pot, the recommended dose per seed mass was converted to the pot scale. The dose of 200 mL/50 kg is equivalent to 4 mL of Rizokop per kilogram of seeds (0.004 mL/g). Assuming an average mass of 0.17 g per seed (thousand-seed weight of 170 g, a reference value for soybean cultivars with a similar growth cycle), the three seeds in each pot total about 0.51 g and correspond to approximately 0.002 mL (2 µL) of the inoculant per pot.

Since this volume is too small to be accurately measured per pot, the treatment was performed in batches: 50 seeds (about 8.5 g) received 34 µL of Rizokop, measured with a micropipette, which covers the 45 seeds in the inoculated treatments (T2, T4, and T6; 15 seeds per treatment) with a safety margin. The total volume of liquid applied to the batch did not exceed 51 µL (limit of 6 mL/kg), so that dilution with water, when necessary, was limited to approximately 17 µL.

According to the usage guidelines provided by Koppert Brasil (2026), Rizokop should be the last product applied to the seeds; the seeds should be allowed to dry in the shade and sown within 24 hours of inoculation. In areas under first-time cultivation, it is also recommended to avoid treating the seeds with products other than the inoculant, as fungicides, insecticides, and micronutrients can reduce bacterial survival—an effect that is more common in these soils, where the rhizobium population is low (Hungria; Nogueira, 2020).

Considering the reference water potential of 5 to 7 mm/day, water regimes were induced by controlling the amount of water applied. To convert the water column into volume per pot, the reported inner radius of 10 cm was used, corresponding to a surface area of 314.16 cm^2. Since 1 mm of water column is equivalent to 0.1 cm of water height, each 1 mm/day corresponds to 31.42 mL per pot. Thus, approximately 62.8 mL/pot/day was applied in the deficit treatment (2 mm/day), 157.1 to 219.9 mL/pot/day under ideal conditions (5 to 7 mm/day), and 314.2 mL/pot/day in the excess treatment (10 mm/day). The conversion was

performed by the authors based on the surface area of the pots, using the following formula: volume (mL) = thickness (mm) × pot area (cm²) × 0.1.

Watering was performed manually using watering cans, with the calculated volumes for each pot applied daily according to the respective treatment. The treatments were conducted under the natural thermal conditions of Porto Nacional (TO), without any temperature manipulation.

To characterize the thermal conditions of the experimental environment in Porto Nacional (TO), daily meteorological records from the National Institute of Meteorology (INMET) were used, obtained from the historical data archive covering the period from August 26 to September 28, 2026. The daily maximum and minimum temperatures recorded at the weather station—whose data are included in the archive provided by INMET—were considered. The daily average temperature presented was estimated by the authors using the arithmetic mean of the maximum and minimum temperatures (Table 2) and does not correspond to a daily average calculated from all hourly observations.

Table 2 – Estimated minimum, maximum, and average air temperatures in Porto Nacional (TO), from August 26 to September 28, 2026.

Date	Lowest temperature (°C)	Highest temperature (°C)	Estimated average temperature (°C)
26/08	22,9	38,8	30,8
27/08	22,8	38,2	30,5
28/08	23,1	38,9	31,0
29/08	23,6	39,8	31,7
30/08	25,6	40,0	32,8
31/08	25,1	40,2	32,7
01/09	23,1	39,5	31,3
02/09	24,1	40,1	32,1
03/09	25,7	39,1	32,4
04/09	26,8	32,1	29,5
05/09	24,4	37,5	30,9
06/09	25,1	36,1	30,6
07/09	24,3	38,2	31,2
08/09	24,7	35,8	30,2
09/09	23,3	38,9	31,1
10/09	23,1	39,6	31,4
11/09	23,3	41,1	32,2
12/09	23,1	40,1	31,6
13/09	22,9	40,7	31,8
14/09	24,8	39,1	32,0

15/09	25,3	39,7	32,5
16/09	26,0	39,9	33,0
17/09	23,8	40,2	32,0
18/09	23,8	41,3	32,5
19/09	25,1	41,1	33,1
20/09	24,9	39,2	32,0
21/09	22,9	39,7	31,3
22/09	24,6	41,1	32,9
23/09	26,8	40,7	33,8
24/09	26,5	40,5	33,5
25/09	27,0	32,6	29,8
26/09	24,5	37,8	31,1
27/09	26,1	41,1	33,6
28/09	26,9	40,4	33,6

Source: Data from INMET (2026), compiled by the authors.

Note: Minimum and maximum temperatures correspond to the daily values provided in the INMET dataset. Average temperature estimated by the authors = (minimum temperature + maximum temperature) / 2. Values are presented to one decimal place.

No instrumental measurements of soil temperature were taken in the pots. For this reason, no soil temperature table is presented, as air temperature should not be used as a direct substitute for substrate temperature.

The data presented in Table 2 characterize the atmospheric temperature conditions recorded during the period in question. Since there was no continuous monitoring of substrate temperature in the pots, it is not possible to state that the air temperature values represent the temperature actually experienced by the roots.

2.5 VARIABLES EVALUATED

The variables described below were evaluated. Emergence and IVE were determined in the 30 pots. The number and diameter of nodules, root length, and fresh root mass were measured in all plants that remained in the pots and were available for evaluation at the V5 stage, totaling 14 plants; no sample selection of individuals was performed.

a) Emerged plants (%): emergence was assessed by visual inspection on August 29, 2026, and August 30, 2026, corresponding to the third and fourth days after sowing (DAS). The number of emerged seedlings was considered relative to the total number of seeds sown, according to Equation 1, with results expressed as a percentage. The seedlings were classified according to the criteria described by Marcos-Filho (2015).

$$\text{Germination (\%)} = (\text{emerged seedlings} / \text{seeds sown}) \times 100 \qquad (1)$$

b) Germination rate index (GRI): determined by counting the number of emerged seedlings on each evaluation date and calculated according to the principle proposed by Maguire (1962), as shown in Equation 2.

$$EER = N1/D1 + N2/D2 + ... + Nn/Dn \qquad (2)$$

where Ni is the number of emerged seedlings at assessment i and Di is the number of days from sowing to assessment i. In this study, D1 = 3 (August 29) and D2 = 4 (August 30). The EER was calculated for each pot.

c) Number of nodules: determined by direct manual counting of the nodules present in the root system of each evaluated plant, as an indicator of the establishment of symbiosis between the plant and nitrogen-fixing bacteria, according to the methodology described by Hungria, Campo, and Mendes (2001).

d) Nodulation efficiency: evaluated based on nodule formation and development. The diameter of each nodule was measured with a caliper, and the nodules were grouped into 1-mm classes, from which the weighted average diameter per treatment was calculated, following the methodological criteria proposed by Hungria, Campo, and Mendes (2001).

e) Root length (cm) and fresh root mass (g): Root length was determined using a graduated ruler by measuring the root system of the evaluated plants. Fresh root mass was obtained by weighing on a precision scale. These parameters were used as indicators of initial root growth, as described by Marcos-Filho (2015).

2.6 STATISTICAL ANALYSIS

The data were subjected to one-way analysis of variance (ANOVA) for a single factor (treatment), and the means were compared using Tukey's test at a 5% significance level. The results are expressed as mean ± standard deviation (SD).

For emergence and IVE, the experimental unit was the pot (five replicates per treatment). For the number of nodules, root length, and fresh root mass, all plants available for evaluation at the V5 stage were considered, totaling 14 individuals (T1: 2; T2: 4; T3: 2; T4: 2; T5: 2; T6: 2). The difference in the number of plants evaluated resulted from the emergence and establishment observed in each treatment, not from the researchers' selection of individuals. Given the small and uneven number of plants evaluated, the results for these variables were interpreted with caution.

3 RESULTS AND DISCUSSION

For emergence and IVE, all 30 pots were considered. For nodulation and root variables, all 14 plants available at the V5 stage were evaluated (T1: 2; T2: 4; T3: 2; T4: 2; T5: 2; T6: 2), without selecting specific plants within the treatments. Since this was a pot experiment with a small and uneven number of plants evaluated, interpretations were made with caution.

3.1 EMERGENCE AND EMERGENCE RATE INDEX

In each treatment, 15 seeds were sown (five pots with three seeds each). The first seedlings were observed on August 29, 2026 (3 DAS), and the rest on August 30, 2026 (4 DAS) (Table 4). In all treatments, thinning was performed after emergence, leaving one plant per pot.

Emergence ranged from 13.3% (T6) to 60.0% (T2), and the average emergence rate per pot ranged from 0.133 (T6) to 0.533 (T2). There was no significant difference among the treatments for emergence ($F = 1.77$; $p = 0.157$) or for IVE ($F = 1.42$; $p = 0.254$). When the treatments were grouped together, 19 out of 45 seeds germinated in the pots treated with Rizokop (42.2%) and 14 out of 45 in the pots without Rizokop (31.1%).

Table 4 – Emerged seedlings, emergence rate (%) and emergence rate index (ERI) by treatment

Treatment	August 29 (3 DAS)	August 30 (4 DAS)	Total (of 15)	Emergency (%)	IVE per vase
T1	2	1	3	20,0 ± 29,8 a	0,183 ± 0,291 a
T2	5	4	9	60,0 ± 43,5 a	0,533 ± 0,402 a
T3	2	6	8	53,3 ± 38,0 a	0,433 ± 0,308 a
T4	4	4	8	53,3 ± 44,7 a	0,467 ± 0,402 a
T5	3	0	3	20,0 ± 29,8 a	0,200 ± 0,298 a
T6	2	0	2	13,3 ± 18,3 a	0,133 ± 0,183 a

Source: Developed by the authors (2026).

Note: Means ± SD (n = 5 pots); values in the same column marked with the same letter do not differ significantly according to Tukey's test ($p < 0.05$). DAS = days after sowing.

Emergence did not differ among the treatments and was low in most of them, including the control under ideal water conditions (T1, 20.0%). This result is consistent with the fact that inoculation with Bradyrhizobium acts primarily on the symbiosis established after emergence, rather than on germination itself.

At this stage, growth is sustained by seed reserves (Taiz *et al.*, 2017), and the contribution of biological nitrogen fixation to crop development (Hungria; Campo; Mendes, 2007) occurs later, with the formation of nodules. Furthermore, Marcos-Filho (2015) points out that emergence depends on the physiological quality of the seeds and environmental conditions, suggesting that factors unrelated to the treatments may have influenced the results.

Among these factors, temperature stands out. Vital *et al.* (2022) emphasize that fluctuations in water and temperature increase physiological risks to soybeans, especially during germination, and Guan *et al.* (2022) report that excessive heat reduces germination. It is therefore plausible that the region's natural temperatures (Table 2) contributed to the low percentages observed, although their effect cannot be isolated, since all treatments were subjected to the same thermal conditions.

Rocha *et al.* (2024) report improvements in the early development of soybeans with seed inoculation, an effect that was not confirmed for emergence in this trial, possibly due to the high variation among pots. In this regard, T2 numerically showed the highest IVE and the highest emergence rate, but with a high standard deviation, which prevents attributing this difference to the inoculant.

The treatments with excess water (T5 and T6) were among those with the lowest emergence rates (20.0% and 13.3%), which may be related to the oxygen limitation described by Fageria, Baligar, and Clark (2006) for saturated soils. However, T1 also showed 20.0%, and the experimental design does not allow for confirmation of this cause.

3.2 NODULATION AND BIOLOGICAL NITROGEN FIXATION

The number of nodules per plant differed among treatments ($F = 14.67$; $p < 0.001$) (Table 5). The highest mean was observed in T6 (62.0 nodules), followed by T4 (32.5) and T2 (25.3). No nodulation occurred in the T3 plants, and in T1, one of the two plants did not develop nodules.

When the treatments were grouped together, the inoculated plants had a mean of 36.3 nodules per plant ($n = 8$), compared to 4.8 in the non-inoculated plants ($n = 6$); all inoculated plants formed nodules (8 out of 8), compared to 3 out of 6 (50%) in the non-inoculated plants. Within each irrigation regime, the inoculated plants outperformed the non-inoculated plants, with a significant difference between T4 and T3 and between T6 and T5, but not between T2 and T1.

Table 5 – Number of nodules per plant and weighted average nodule diameter by treatment

Treatment	Plants (n)	Nodules per plant (mean ± SD)	Minimum – maximum	Average diameter (mm)
T1	2	4,0 ± 5,7 bc	0 – 8	4,4
T2	4	25,3 ± 7,1 bc	18 – 35	2,8
T3	2	0,0 ± 0,0 c	0	—
T4	2	32,5 ± 10,6 ab	25 – 40	2,4
T5	2	10,5 ± 9,2 bc	4 – 17	3,1
T6	2	62,0 ± 14,1 a	52 – 72	2,7

Source: Developed by the authors (2026).

Note: Means followed by the same letter do not differ according to Tukey's test ($p < 0.05$). — = data not available (no nodulation).

In terms of size, the nodules on the inoculated plants were concentrated in the 2-mm and 3-mm size classes, while the nodules on the uninoculated plants were, on average, larger (Table 6). The weighted average diameter was 4.4 mm in T1 and 3.1 mm in T5, compared to 2.8 mm in T2, 2.4 mm in T4, and 2.7 mm in T6.

Table 6 – Distribution of the number of nodules by diameter class and by treatment (total of plants evaluated).

Treatment	1 mm	2 mm	3 mm	4 mm	5 mm	6 mm	Total
T1	0	0	1	4	2	1	8
T2	6	34	35	23	0	2	100
T3	0	0	0	0	0	0	0
T4	9	29	20	7	0	0	65
T5	0	8	5	6	2	0	21
T6	12	43	41	26	0	2	124

Source: Developed by the authors (2026).

Note: In T2, one of the plants had 18 counted nodules, of which 17 were classified by diameter; the total in Table 6 refers to the classified nodules.

The absence of nodulation in both T3 plants (water deficit without inoculation) is consistent with Hungria and Vargas (2000), who reported that conditions such as water deficit can impair nodule formation and the activity of symbiotic bacteria.

Under the same water deficit, the inoculated plants (T4) formed an average of 32.5 nodules, suggesting that inoculation with Rizokop helped maintain the symbiosis under these conditions. A similar result was reported by Jabborova *et al.* (2021), who observed sustained nodulation and growth under drought conditions in co-inoculated plants, although in that

study the inoculation involved more than one bacterium and, therefore, the comparison is not direct.

Under waterlogged conditions, lower nodulation was expected, since, according to Fageria, Baligar, and Clark (2006), hypoxia limits root growth and interferes with biological nitrogen fixation. In the present experiment, however, T6 had the highest average number of nodules (62.0), and T5 outnumbered T1.

It is possible that the 10 mm/day irrigation regime, applied to 10-liter pots, did not cause prolonged hypoxia and that the growth stage evaluated (up to V5) was too early to manifest this effect. Furthermore, T6 consisted of only two plants, with quite distinct values (52 and 72 nodules), which limits the generalizability of the results.

The presence of nodules on uninoculated plants (T1 and T5) indicates that the control treatment was not free of nitrogen-fixing bacteria. The most likely explanation is contamination of the substrate by Bradyrhizobium, since the soil used was collected at the research site itself and was not sterilized, allowing the bacterial populations present there to form nodules on the plants without inoculation.

This hypothesis is consistent with the literature. Soybeans have been cultivated for decades in Brazil, and the cultivation of inoculated soybeans increases the population of rhizobia in the soil (Thies; Woomer; Singleton, 1995; Zilli *et al.*, 2013), with soils frequently harboring established populations capable of nodulating uninoculated plants (Campos; Hungria; Tedesco, 2001; Mendes; Hungria; Vargas, 2003).

In Cerrado Latossolo initially devoid of soybean rhizobia, Mendes, Hungria, and Vargas (2004) found that introduced strains dispersed throughout the area and came to occupy more than half of the nodules even where they had never been inoculated. Zilli *et al.* (2021) recorded, in areas previously cultivated with inoculated soybeans, populations of bradyrhizobia of at least 10^3 cells per gram of soil, which reduces the response to inoculation.

Cured cattle manure added to the substrate may have further promoted this nodulation. Silva, Raimundo, and Forti (2019) reported that, in pots containing cattle manure, the number of nodules was similar in inoculated and non-inoculated plants (10.8 and 8.9 nodules per plant), which they attributed to improved physical, chemical, and biological soil conditions.

Since the soil was the same across all treatments, the absence of nodules in T3 suggests that water deficit limited infection by native rhizobia, which is consistent with Hungria and Vargas (2000). Cross-contamination from splashing during manual irrigation cannot be entirely ruled out, but the presence of the bacteria in the soil itself already explains the nodulation observed in the controls.

The origin of the nodules was not confirmed by strain isolation or by bait plant testing. Since the control was partially nodulated, the difference between inoculated and non-inoculated plants may be underestimated. In future trials, it is recommended to quantify the soil rhizobium population, use sterilized substrate, and water each pot individually.

The nodules on the uninoculated plants were, on average, larger, while those on the inoculated plants were more numerous and smaller. Based on these observations, it seems possible to infer that inoculation promoted the formation of many developing nodules, but this interpretation needs to be confirmed by assessing the dry weight of the nodules and their activity.

The greater nodulation in inoculated plants is consistent with the importance of Bradyrhizobium-based inoculants for biological nitrogen fixation in soybeans (Hungria; Campo; Mendes, 2007; Silva *et al.*, 2024), although fixation efficiency was not measured in this study.

3.3 ROOT LENGTH AND FRESH ROOT WEIGHT

Root length differed among treatments ($F = 25.35$; $p < 0.001$), with higher means in T6 (36.0 cm) and T2 (34.3 cm) and a lower mean in T3 (23.5 cm) (Table 7 and figure 2). When the treatments were grouped together, the mean was 33.4 cm for inoculated plants and 27.8 cm for non-inoculated plants.

Compared to the respective non-inoculated control, root length was 20.2% greater in T2, 23.4% greater in T4, and 14.3% greater in T6; the difference was significant between T2 and T1 and between T4 and T3, but not between T6 and T5.

Fresh root mass also differed among the treatments ($F = 5.08$; $p = 0.022$), with the highest mean in T6 (7.54 g) and the lowest in T3 (1.58 g). The mean for T6 consists of two plants with masses of 4.88 g and 10.20 g, resulting in a high standard deviation (3.76 g).

The pooled means were 4.12 g for the inoculated plants and 1.86 g for the non-inoculated plants. Compared to the respective control, fresh mass was about 40% higher in T2, 92% higher in T4, and 295% higher in T6, but only the difference between T6 and T5 was significant.

Table 7 – Root length and fresh root weight by treatment

Treatment	Plants (n)	Root length (cm)	Fresh root weight (g)
T1	2	28,5 ± 0,7 b	2,10 ± 0,07 b
T2	4	34,3 ± 1,3 a	2,94 ± 0,44 b
T3	2	23,5 ± 2,1 c	1,58 ± 0,06 b

T4	2	29,0 ± 1,4 b	3,04 ± 0,59 ab
T5	2	31,5 ± 0,7 ab	1,91 ± 0,57 b
T6	2	36,0 ± 1,4 a	7,54 ± 3,76 a

Source: Developed by the authors (2026).

Note: Means ± SD followed by the same letter in the same column do not differ according to Tukey's test ($p < 0.05$).

The inoculated plants had longer roots under all water regimes, and the shortest average root length occurred in T3, under water deficit without inoculation—a condition in which water deficiency restricts soybean growth (Sinclair; Vadez, 2012). This finding is consistent with Fageria, Baligar, and Clark (2006), who associate water stress with reduced root growth.

It is also consistent with Vessey (2003) and Bhattacharyya and Jha (2012), who describe the stimulation of the root system by root-associated microorganisms. The evidence suggests that Rizokop helped mitigate the reduction in root length under water deficit conditions (T4 compared to T3), consistent with the effects of beneficial bacteria on root development reported by Jabborova *et al.* (2021).

It should be noted that the efficiency of biological nitrogen fixation was not measured in this study. Thus, the increased nitrogen supply—which Taiz *et al.* (2017) describe as favorable for plant growth—is merely a hypothesis to explain the greater root length, and conclusions regarding biological nitrogen fixation are limited.

Fresh root mass showed the same general pattern, but was strongly influenced by a single plant in the T6 treatment (10.20 g). Since fresh mass includes water retained in the tissues—which tends to be higher under greater water availability—it is not equivalent to biomass accumulation; therefore, it is recommended that dry mass be determined in future experiments.

3.4 MITIGATION OF ABIOTIC STRESSES AND LIMITATIONS OF THE STUDY

Overall, the results of this experiment suggest that Rizokop mitigated the effects of water deficit on nodulation and root length. This interpretation should be considered indicative, as it stems from the small number of plants evaluated and the specific conditions of the experiment. Under waterlogged conditions, the effect on nodulation was significant, but there was no clear improvement in emergence, and the effect on the roots was less pronounced.

Heat stress, in turn, could not be evaluated, as all treatments were subjected to the same ambient temperature (Table 2), with no thermal control treatment and no temperature measurements taken in the pots; the data presented merely characterize the experimental environment. Since high temperatures alter enzymatic activity and the water balance of plants (Hatfield; Prueger, 2015), this factor should be included in future trials.

Among the study's limitations is the small number of plants evaluated per treatment (two, except in T2), which created an imbalance among treatments, gave excessive weight to a very high fresh mass value in T6, and prevented verification of the assumptions of the analysis of variance. Therefore, the statistical results should be considered indicative.

Regarding the substrate, the soil was not sterilized, the native rhizobium population was not quantified, and the nutrient contribution from cattle manure was not considered in the calculations for liming and fertilization. As for the variables, fresh root mass was used without determining dry mass, and the efficiency of biological nitrogen fixation was not measured.

Regarding the environment, substrate temperature and humidity in the pots were not measured. Table 2 presents only the air temperature records provided by INMET; therefore, it does not allow for a direct estimation of soil or substrate temperature.

Furthermore, the experiment was limited to the V5 stage and a single cultivar, making it impossible to draw conclusions about yield. The biochemical mechanisms of cellular protection described by Wang *et al.* (2022), such as the activation of antioxidant enzymes, were also not evaluated; thus, the attenuation of stresses suggested here refers only to the effects on nodulation and roots.

As a suggestion for future research, it is recommended to increase the number of replicates and plants evaluated, monitor temperature and humidity, determine the dry weight of roots and nodules, assess biological nitrogen fixation activity, and validate the results in the field.

4 CONCLUSION

Under the conditions of this experiment, Rizokop did not significantly alter soybean emergence or the emergence rate index, but it promoted nodulation and early root growth.

Under water deficit conditions, only the inoculated plants formed nodules, suggesting that the product helped mitigate this stress; under waterlogged conditions, the effect was limited to nodulation. The effect of heat stress could not be evaluated, as all treatments were

subjected to the same natural temperature. As a hypothetical practical implication, in soybean-growing areas subject to periods of water stress during establishment, the use of Rizokop could be considered as a complementary strategy to promote nodule formation and early root development. This possibility, however, needs to be validated in field trials with a greater number of replicates, monitoring of soil moisture, and evaluation of the efficiency of biological nitrogen fixation; based on the data from this study, it is not possible to assert that the product guarantees increased productivity or eliminates the effects of drought.

The results should be interpreted with caution due to the small number of plants evaluated, low emergence in some treatments, the presence of nodules on non-inoculated plants—attributed to native soil rhizobia—and the short evaluation period. Further trials are recommended with more replicates, using sterilized substrate or substrate with a known rhizobium population, recording environmental conditions, assessing dry matter, and conducting field validation.

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