Interaction of soybean seedlings with beneficial rhizobacteria

Interação de plântulas de soja com rizobactérias benéficas

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Highlights

Beneficial microorganisms were evaluated in the development of soybean seedlings.
The co-inoculation of *Serratia marcescens* + Bacillus spp. is significant.
Beneficial microorganisms influenced the root development of soybeans.

Abstract

Beneficial rhizobacteria are multifunctional microorganisms that stimulate plant growth through direct mechanisms such as the solubilization of phosphorus and potassium present in soil minerals, biological nitrogen fixation and production of plant hormones. The objective of this study was to determine the effect of beneficial rhizobacteria on the initial development, promoting growth and resistance of soybean seedlings, aiming not only for increased production but also for agricultural sustainability through the maximization of biological efficiency in the early development of soybean seedlings. The experiment, under controlled conditions, was conducted in a completely randomized design, with four treatments and ten replications. The treatments consisted in the microbiolization of soybean seeds with: 1. Control (without microorganism); 2. *Serratia marcenses* (BRM 32114); 3. *Bacillus* spp. (BRM63573) and 4. BRM 32114 + (BRM63573). After the microbiolization of the seeds, they were sown and placed to germinate, after 12 days, the seedlings were harvested and divided into shoots and roots. The roots were washed and photographed with digital camera. The images were analyzed and determined length, diameter, surface area and volume of roots. Then, root and shoot were dried and weighed for biomass determination. Co-inculation, BRM 32114+ BRM63573, promoted greater robustness of the root system of soybean seedlings with increased length (16.2%), diameter (22.5%), volume (43.4%) and dry biomass (29.7%). The

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dry matter of shoots and total soybean seedlings was superior to the control treatment in all treatments with rhizobacteria. Therefore, plant growth promoting rhizobacteria are considered promising technology to provide greater development of soybean seedlings.

Key words: *Glycine max* L. Growth promotion. Rhizobacteria. Sustainability.

Resumo

Rizobactérias benéficas são microrganismos multifuncionais que estimulam o crescimento das plantas por meio de mecanismos diretos como a solubilização de fósforo e potássio presentes nos minerais do solo, fixação biológica de nitrogênio e produção de hormônios vegetais. O objetivo do trabalho foi determinar o efeito do uso de rizobactérias benéficas no desenvolvimento inicial na promoção do crescimento e resistência de plântulas de soja, visando não apenas o aumento da produção, mas também a sustentabilidade agrícola através da maximização da eficiência biológica no desenvolvimento inicial das de plântulas de soja. O experimento, em condições controladas, foi conduzido em delineamento inteiramente casualizado, com quatro tratamentos e dez repetições. Os tratamentos consistiram na microbiolização de sementes de soja com: 1. Controle (sem microrganismo); 2. *Serratia marcenses* (BRM 32114); 3. *Bacillus* spp. (BRM63573) e 4. BRM 32114 + (BRM63573). Após a microbiolização, as sementes foram colocadas para germinar e, após 12 dias, as mudas foram colhidas e divididas em parte aérea e raízes. As raízes foram lavadas e fotografadas com câmera digital. As imagens foram analisadas e determinou-se comprimento, diâmetro, área superficial e volume de raízes. Em seguida, raiz e parte aérea foram secas e pesadas para determinação de biomassa. A co-inoculação com BRM 32114+ BRM63573 promoveu maior robustez do sistema radicular de plântulas de soja com aumento do comprimento (16,2%), diâmetro (22,5%), volume (43,4%) e biomassa seca (29,7%). A matéria seca de parte aérea e total de plântulas de soja foi superior ao tratamento controle em todos os tratamentos com rizobactérias. Portanto, rizobactérias promotoras de crescimento vegetal são consideradas tecnologia promissora para proporcionar maior desenvolvimento de plântulas de soja.

Palavras-chave: *Glycine max* L. Promoção de crescimento. Rizobactéria. Sustentabilidade.

Introduction

The soybean crop (*Glycine max* (L.) Merr.) holds significant socio-economic value worldwide due to its crucial role in both human and animal nutrition, as well as its use as a raw material for biofuel production (Mattos & Caires, 2022). In the 2020/21 growing season, Brazil emerged as the leading soybean producer (139.50 million tons), followed by the United States (114.75 million tons) and Argentina (46.20 million tons) (United States Department of Agriculture [USDA], 2022).

Soybean cultivation in Brazil is characterized by the excessive use of inputs, particularly phosphorus and potassium-rich fertilizers, insecticides, fungicides, and herbicides. Consequently, production costs are high, leading to a reduced profitability of the crop and environmental contamination. In this context, the exploration of technological alternatives that reduce the reliance on synthetic inputs and environmental contamination has become the focus of numerous research endeavors (Ballabio et al., 2013).

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The utilization of multifunctional and beneficial microorganisms emerges as one of the most promising technologies for the sustainable intensification of agriculture (Lopes & Albuquerque, 2018). Plant growth-promoting microorganisms can be either endophytic, colonizing plant tissues such as xylem or root cortex without causing harm or displaying external structures, or rhizospheric, comprising the microbial community residing in the region near the roots and benefiting from plant exudates (Cardoso & Andreote, 2016).

Rhizobacteria, as multifunctional microorganisms, generally confer various benefits to plants, including the production of phytohormones (gibberellins, auxins, and cytokinins) (Santoyo et al., 2016), antibiotics, and siderophores (Abbmondi et al., 2016). They contribute to biological nitrogen fixation (Pacentchuck et al., 2020), inorganic phosphate solubilization, and organic phosphate mineralization, as well as inhibition of ethylene production (Santoyo et al., 2016), and mitigation of oxidative, saline, and water stresses. Recent studies have demonstrated the beneficial effects of multifunctional microorganisms in crops such as rice (Fernandes et al., 2021), maize (Cruz et al., 2022), common beans (Rezende et al., 2021), soybeans (Chagas et al., 2022), among others.

Microorganisms can also contribute to the initial development of agricultural crops, with positive repercussions on grain productivity (Fernandes et al., 2021). However, there is still a limited number of studies on the use of microorganisms in the initial growth of soybeans. Therefore, the objective of this study was to determine the effect of beneficial rhizobacteria on the initial development of soybean seedlings.

Materials and Methods

The experiment was conducted in the greenhouse facilities of Embrapa Rice and Beans/Santo Antônio de Goiás, GO. The experimental design employed was completely randomized, with four treatments and ten replications, totaling 40 plots. The treatments involved the microbiolization of soybean seeds, cultivar BRS 6970IPRO, with isolated or consortium rhizobacteria. Thus, the treatments were: 1. Control (without microorganisms); 2. *Serratia marcescens* (BRM 32114); 3. *Bacillus* spp. (BRM63573); and 4. *Serratia marcescens* (BRM 32114) + *Bacillus* spp. (BRM63573) (Figure 1). These rhizobacteria are stored and preserved in the Multifunctional Microorganisms Collection at Embrapa Rice and Beans.

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Figure 1. Images of soybean seedlings after seed microbiolization:
1. Control (without microorganism); 2. *Serratia marcescens* (BRM 32114); 3. *Bacillus* spp. (BRM 63573); and 4. *Serratia marcescens* (BRM 32114) + *Bacillus* spp. (BRM 63573).

For soybean seed microbiolization, the seeds were immersed in bacterial suspensions (Filippi et al., 2011) produced by cultivating the mentioned rhizobacteria on solid medium (nutrient agar). The suspension preparation was carried out in liquid medium 523 (nutrient broth) in a shaking incubator for 24 hours at 28°C (Kado & Heskett, 1970). Subsequently, the concentration was fixed at A540 = 0.5 using a spectrophotometer (108 CFU, colony-forming units). Before microbiolization, the seeds were disinfested by immersion in 0.05% sodium hypochlorite for two minutes and then in 70% alcohol for the same duration, followed by air-drying at room temperature (29°C). Soybean seeds were immersed in each rhizobacterial suspension and autoclaved for 4 hours with constant agitation at 25°C. Control seeds were immersed in distilled water for the same period (4 h).

For each experimental unit, 500 mL plastic cups filled with medium-textured ravine Oxisol from the surface layer (0 - 0.20 m) were used, where two seeds were sown per cup. Daily irrigations were performed, maintaining soil moisture at field capacity. Additionally, no fertilization or inoculation

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with Rhizobium occurred. After 12 days, soybean seedlings were removed from the cups and photographed using a digital camera (Figure 1).

Subsequently, the aboveground part of the seedlings was detached, placed in labeled paper bags, and taken to a forced ventilation oven (65°C) until a constant mass was achieved. The roots were washed, arranged on a horizontal support, and photographed. The obtained images were analyzed using WinRHIZO 2012 software, Regent Instruments, Inc., Quebec City, QC, Canada (Arsenault et al., 1995), and the root parameters length (LengR, cm), diameter (DiamR, mm), surface area (AreaS, cm2), and volume (VolR, cm3) were determined.

After determining the root parameters in WinRhizo, the roots were placed in labeled paper bags and taken to a forced ventilation oven at 65°C until a constant mass was achieved. The material in each bag was weighed to determine the dry mass of roots (MSR), aboveground part (MSPA), and total (root + aboveground) (MST) using a precision scale. The obtained data were subjected to analysis of variance using the statistical software Sisvar 5.6, and when significant, means were compared using the LSD test (p≤0.05).

Principal components (PCs) were used as response variables when the correlation test result produced r ≥ 0.50. The biplot (two-dimensional graph) correlated the isolated and consortium rhizobacteria with the response variables using the R statistical software.

Results and Discussion

The co-inoculation of soybean seeds with *Serratia marcescens* (BRM 32114) + *Bacillus* spp. (BRM63573) significantly stimulated the root growth of soybean seedlings compared to the control treatment (without microorganisms) (Table 1). The increase was approximately 16.2%, 22.5%, and 43.4% for root length, diameter, and volume, respectively. In contrast, the inoculation with isolated rhizobacteria, *Serratia marcescens* (BRM 32114), and *Bacillus* spp. (BRM 63573) did not differ significantly from each other in the root parameters evaluated by WinRhizo. However, these rhizobacteria promoted greater root biomass (average value of 0.043 g) in soybean seedlings compared to the treatment without microorganisms (0.037 g). To overcome some limitations of inoculation and enhance the efficiency of plant growth-promoting rhizobacteria, co-inoculation, defined as the combined application of beneficial microorganisms, aims to increase nodulation rate, favor early and more intense nodulation, improve nutrient and water absorption, and consequently, enhance plant growth and tolerance to adverse environmental conditions (Khan et al., 2020).

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Table 1
Characteristics of the root system of soybean seedlings, cultivar BRS 6970IPRO, inoculated with plant growth-promoting rhizobacteria, both isolated and in consortium. Santo Antônio de Goiás, Goiás

Treatment	Root System			
	LengR (cm)	DiamR (mm)	AreaS (cm²)	VolR (cm³)
Serratia marcenses (BRM 32114) + *Bacillus* spp. (BRM63573)	11,07 a	0,98 a	0,028	0,076 a
Serratia marcenses (BRM 32114)	10,53 ab	0,99 a	0,027	0,077 a
Bacillus spp. (BRM63573)	9,25 b	0,91 ab	0,030	0,071 ab
Control (without microrganism)	9,53 b	0,80 b	0,029	0,053 b
Factor ANOVA (p value)				
Microrganims (M)	0,046	0,047	0,288	0,000
CV (%)	15,55	17,42	12,54	17,27

*Means followed by the same letter do not differ according to the LSD test ($p < 0.05$).
LengR - root length; DiamR - root diameter; AreaS - root surface area; VolR - root volume.

Figure 2 displays images of the root system of soybean seedlings treated with the beneficial rhizobacteria evaluated in this study. Similar results were obtained for the root system of rice and corn treated with *Azospirillum* sp., showing an increase of 86.3% and 43.7%, respectively (Fernandes et al., 2021; Cruz et al., 2022). Both authors suggested modifications induced by the *Azospirillum* sp. rhizobacteria in the root morphology of the studied species, leading to increased water and nutrient absorption rates. In another study, Cruz et al. (2022) observed root system growth in corn seedlings treated with *Serratia* sp., *Bacillus* sp., and *Azospirillum* sp. The authors suggested that root growth was partly due to the production of hormones by the microorganisms.
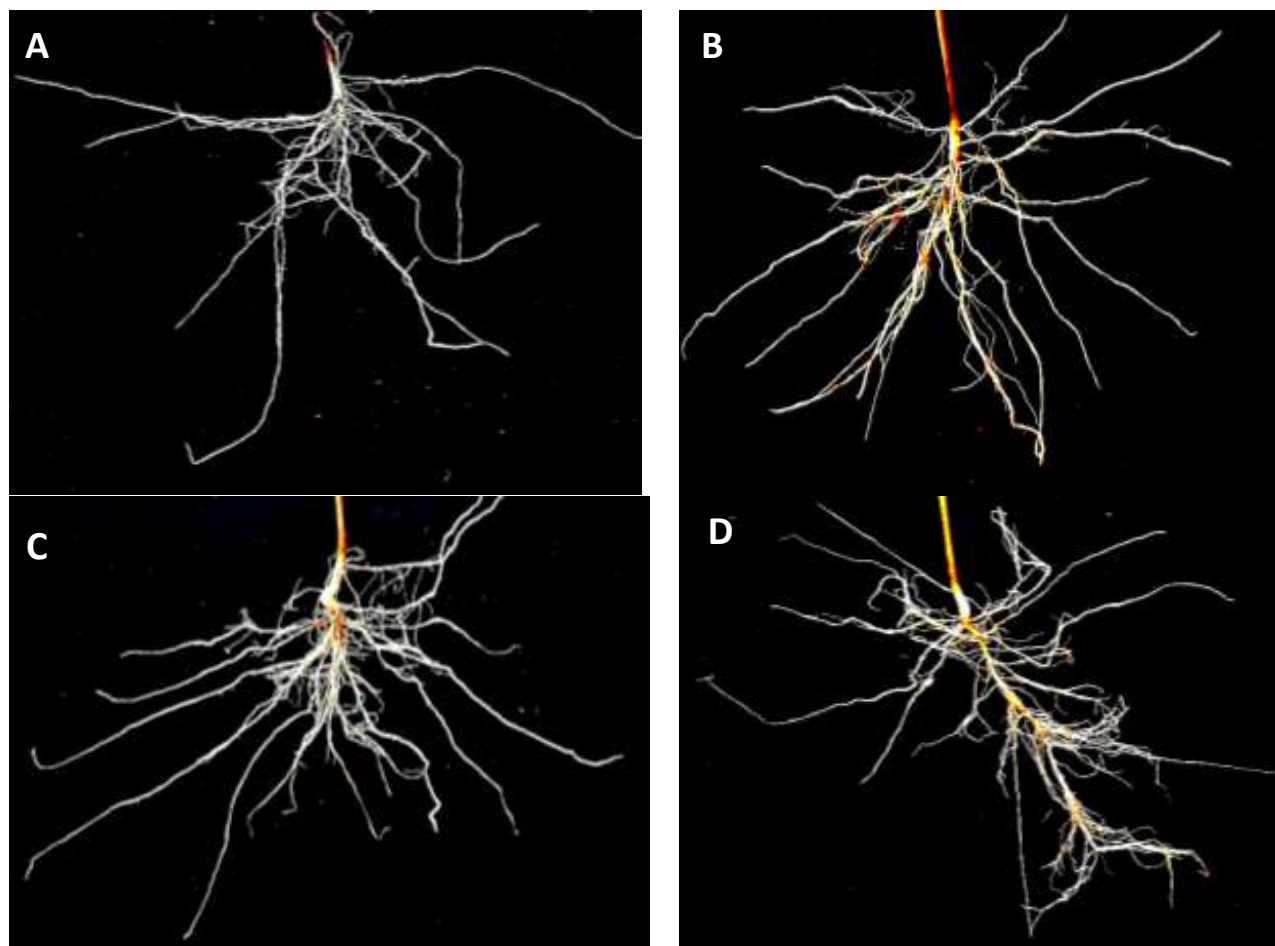
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Figure 2. Root system of soybean seedlings subjected to microbiolization with beneficial rhizobacteria.
A. Control (without microorganisms), B. *Serratia marcescens* (BRM 32114), C. *Bacillus* spp. (BRM 67753), D. *Serratia marcescens* (BRM 32114) + *Bacillus* spp. (BRM 67753). Roots were photographed with a digital camera 12 days after sowing. The images were analyzed using the WinRHIZO 2012 software.

Soybean seedlings treated with Serratia marcescens (BRM 32114) + *Bacillus* spp. (BRM63573), *Serratia marcescens* (BRM 32114), and *Bacillus* spp. (BRM63573) showed a significant increase in total dry matter (MST) of 36.6%, 35.9%, and 32.4%, respectively, and in aboveground dry matter (MSPA) by 39.0%, 43.8%, and 41.0%, respectively, compared to the control treatment (Table 2). *Serratia* genus rhizobacteria belong to the Enterobacteriaceae family, facultative gram-negative bacteria that can be isolated from water, soil, plants, and insects. Serratia is capable of promoting plant growth through the production of phytohormones and siderophores. Seed inoculation with this genus promotes phytoremediation potential, aiding in greater biomass accumulation and increased nutrient absorption rates (Silva et al., 2023). On the other hand,

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Bacillus genus rhizobacteria belong to the Bacilliaceae family and can colonize plant rhizospheres, promoting plant development by playing a fundamental role in nutrient cycling and increasing productive levels, as well as benefiting plants during stress periods (Chagas et al., 2022). Thus, these beneficial growth-promoting characteristics of rhizobacteria significantly affected the development of soybean seedlings.

Table 2
Dry root mass (DRM), dry aboveground mass (DAM), and total dry mass (TDM) of soybean seedlings, cultivar BRS 6970IPRO, inoculated with plant growth-promoting rhizobacteria, both isolated and in consortium. Santo Antônio de Goiás, Goiás

Treatments	MSR	MSPA	MST
Microrganisms (M)	(g)	(g)	(g)
Serratia marcenses (BRM 32114) + *Bacillus* spp. (BRM63573)	0,048 a	0,146 a	0,194 a
Serratia marcenses (BRM 32114)	0,042 a	0,151 a	0.193 a
Bacillus spp. (BRM63573)	0,040 a	0,148 a	0,188 a
Control (without microrganism)	0,037 b	0,105 b	0,142 b
Factor ANOVA (p value)			
Microrganims (M)	0,051	0,052	0,053
CV (%)	29,14	32,11	26,13

*Means followed by the same letter do not differ according to the LSD test (P < 0.05).

Several studies confirm the beneficial effects of microorganisms on plant growth: Tavarez et al. (2019) demonstrated a 7% and 45% increase in root and shoot dry matter in upland rice plants treated with Trichoderma spp. + *Azospirillum* spp., suggesting that root system colonization by microorganisms led to increased root development, resulting in greater water and nutrient absorption by the plants. Cruz et al. (2022) described an increase in the aboveground dry matter of corn seedlings treated with *Azospirillum* sp. (83.13%) and *Bacillus* sp. (63.44%). Chagas et al. (2022) reported that the use of *Bacillus subtilis* led to significant increases (52.10%) in the total dry matter of soybean seedlings. These results may be related to the ability of microorganisms to produce phytohormones and solubilize nutrients. Additionally, seedlings with higher dry matter accumulation signal greater nutrient absorption, directly affecting growth, stress tolerance, and increased productivity potential (Cruz et al., 2022).

Regarding principal component analysis, the root system characteristics (length, diameter, surface area, volume, and dry mass) of soybean seedlings treated with beneficial rhizobacteria, both isolated and in consortium, were best described by two principal components, PC1 (77.7%) and PC2 (24.6%), accounting for 99.3% of the

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data variation (Figure 3). The factorial map (biplot) shows groups of variables (arrows) denoting positive and negative correlations with each principal component (PC), with the arrow's length indicating the magnitude of each response for each PC (Figure 3). This means that PC1 correlated negatively with all analyzed variables, while PC2 correlated positively with root length, area, and dry mass, and negatively with root diameter, volume, and total dry mass and aboveground dry mass of soybean seedlings.
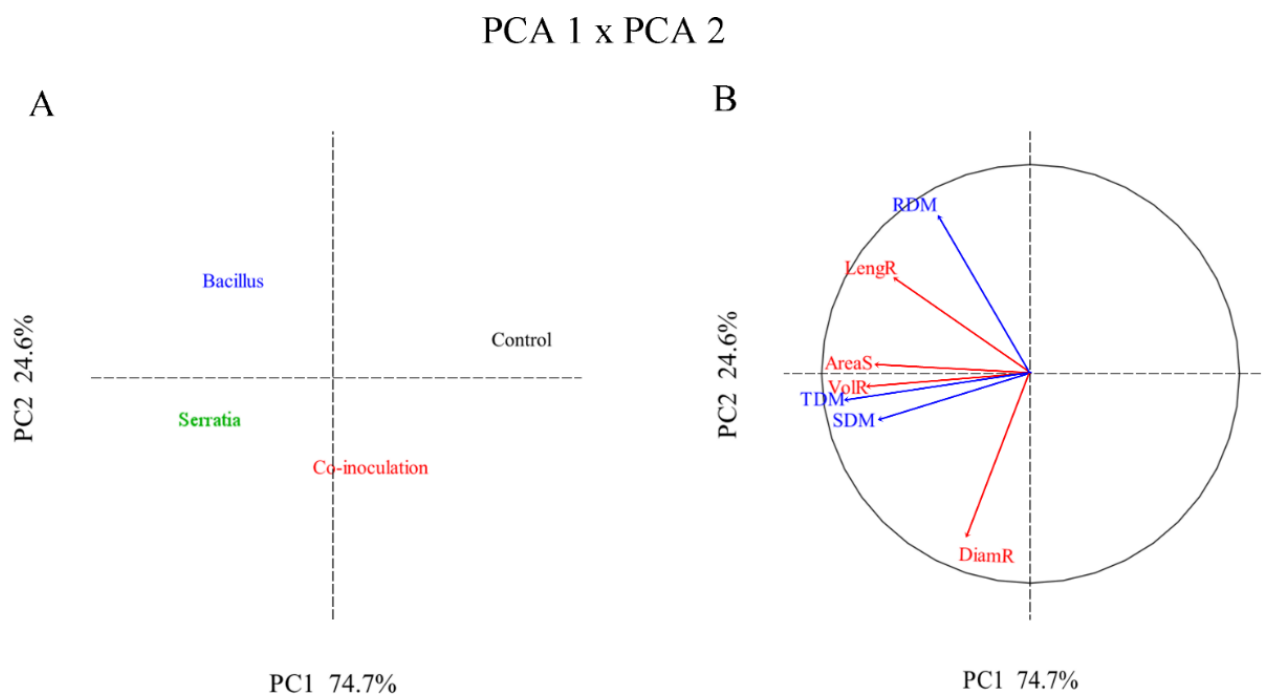
PCA 1 x PCA 2

Figure 3. Principal Component Analysis (PCA) PCA1 X PCA2, explaining the correlations between the evaluated variables and four treatments.
A. Biplot chart for treatments: *Bacillus, Serratia*, Co-inoculation (*Bacillus + Serratia*), and control treatment.
B. Chart with variables or circle of correlation.

Concerning the representational quality of microbial treatments, isolated and consortium rhizobacteria, for the analyzed variables, Serratia marcescens (BRM 32114) showed a higher positive correlation for root volume, total dry mass, root dry mass, and aboveground dry mass (Figure 2). Root length, root dry mass, and root area variables were positively correlated with the *Bacillus* sp. treatment. The use of rhizobacteria from different species may present specific characteristics and functionalities in promoting plant growth (Brunetta et al., 2007). Moreover, the production of hormones by microorganisms near the root meristematic regions promotes cellular elongation/root system growth, and consequently, greater water and nutrient absorption, with a direct

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impact on plant vigor and production (Nascente et al., 2017).

Therefore, the use of beneficial microorganisms can be considered an innovative technology that promotes greater initial development of soybean seedlings and may have a significant effect on crop grain productivity. Sustainable practices, such as these, can favor the reduction of negative environmental impacts as well as a decrease in production costs (Paiva et al., 2022).

Conclusion

The co-inoculation of BRM 32114 + BRM63573 promoted greater robustness of the root system in soybean seedlings, with an increase in length (16.2%), diameter (22.5%), volume (43.4%), and dry biomass (29.7%). The dry matter of the aboveground and total parts of soybean seedlings was higher than the control treatment in all rhizobacteria treatments.

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