

Multifunctional rhizobacteria in the initial root growth of soybean, common bean, maize, and upland rice seedlings

Rizobactérias multifuncionais no crescimento inicial da raiz de mudas de soja, feijão comum, milho e arroz de terras altas

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Highlights

BRM 63524 stood out in increasing root length across all crops.
Inoculation resulted in greater root dry mass in all the crops evaluated.
The control treatment showed inferior performance in all variables evaluated.
All crops demonstrated an increase in root dry mass with inoculation.

Abstract

Multifunctional rhizobacteria can enhance root development and contribute to crop grain yields. This study aimed to determine the effects of rhizobacteria on the root growth of soybean, common bean, maize, and upland rice seedlings. Separate experiments were conducted for each crop under controlled conditions using a completely randomized design with four treatments and ten replicates. The treatments across the four experiments involved seed microembolization with rhizobacteria isolates: 1. BRM 32111

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(*Burkholderia* sp.), 2. BRM 63523 (*Serratia* sp.), 3. BRM 63524 (*Bacillus* sp.), and a control treatment (without microorganisms). Each experimental unit consisted of 500 mL plastic cups filled with soil and sand and planted with two seeds. After 14 days, the roots of the seedlings from the studied crops were removed from the cups, washed, and photographed using a digital camera. The images were analyzed using the WinRHIZO 2012 software to measure total root length, diameter, surface area, and root volume. Subsequently, the roots were placed in a drying oven and weighed to determine their dry mass. The study results indicate that multifunctional rhizobacteria promote greater initial root development in soybean, common bean, maize, and upland rice. The treatment with the BRM 63524 isolate resulted in the greatest total root length compared to the control treatment, standing out among the evaluated crops. Seedlings not inoculated with rhizobacteria tend to exhibit inferior root development compared to inoculated ones.

Key words: *Bacillus* sp.. *Burkholderia* sp.. *Serratia* sp.. Sustainability. WinRHIZO.

Resumo

As rizobactérias multifuncionais podem melhorar o desenvolvimento radicular e contribuir para a produtividade de grãos das culturas. Este estudo teve como objetivo determinar os efeitos das rizobactérias no crescimento radicular de plântulas de soja, feijão-comum, milho e arroz de terras altas. Foram conduzidos experimentos separados para cada cultura sob condições controladas, utilizando um delineamento inteiramente casualizado com quatro tratamentos e dez repetições. Os tratamentos nos quatro experimentos envolveram a microembebição das sementes com isolados de rizobactérias: 1. BRM 32111 (*Burkholderia* sp.), 2. BRM 63523 (*Serratia* sp.), 3. BRM 63524 (*Bacillus* sp.) e um tratamento controle (sem microrganismos). Cada unidade experimental consistiu em copos plásticos de 500 mL preenchidos com solo e areia, contendo duas sementes. Após 14 dias, as raízes das plântulas das culturas estudadas foram removidas dos copos, lavadas e fotografadas com uma câmera digital. As imagens foram analisadas utilizando o software WinRHIZO 2012 para medir o comprimento total da raiz, diâmetro, área superficial e volume radicular. Posteriormente, as raízes foram secas em estufa e pesadas para determinar sua massa seca. Os resultados do estudo indicam que as rizobactérias multifuncionais promovem um maior desenvolvimento radicular inicial em soja, feijão-comum, milho e arroz de terras altas. O tratamento com o isolado BRM 63524 resultou no maior comprimento total de raiz em comparação com o tratamento controle, destacando-se entre as culturas avaliadas. Plântulas não inoculadas com rizobactérias tendem a apresentar desenvolvimento radicular inferior em comparação com as inoculadas.

Palavras-chave: *Bacillus* sp.. *Burkholderia* sp.. *Serratia* sp.. Sustentabilidade. WinRHIZO.

Introduction

The Brazilian Cerrado, known as the most biodiverse savanna in the world, covers approximately 24% of the national territory

and is considered one of the most important biomes for agricultural production in Brazil (Instituto Chico Mendes de Conservação da Biodiversidade [ICMBio], 2024). In the Cerrado, grain production accounts for most

agricultural output, with soybean, maize, and common bean standing out as key crops. Additionally, upland rice production has been increasing due to the development of highly productive and adapted cultivars. However, the predominant production model for these crops involves excessive use of pesticides and synthetic fertilizers, leading to serious environmental issues and financial impacts (Cruz et al., 2022).

The excessive use of chemical inputs has raised concerns about environmental contamination, particularly soil and water resource pollution. While fertilization provides benefits, the indiscriminate use of fertilizers, especially mineral ones, can lead to environmental issues such as water contamination through eutrophication, which occurs when excess nutrients are transported out of the agricultural system (Ogino et al., 2023). According to Fernandes et al. (2021), increasing the productivity of these crops while reducing chemical input usage is a challenge, particularly due to biotic and abiotic stresses, such as disease outbreaks, prolonged droughts, nutrient deficiencies, and difficulties with initial establishment and plant growth in no-till farming systems.

Concerns about health and the environment have intensified interest in alternative technologies that sustainably ensure competitive yields and crop protection, promoting long-term ecological balance. In this context, pursuing sustainable technologies, such as bioinputs, becomes essential to ensure agricultural productivity and the conservation of natural resources. Bioinputs can be defined as products and technologies that utilize biological agents, such as fungi, bacteria, plants, insects, and animals, to enhance production in agriculture,

aquaculture, and livestock farming (Amaral et al., 2023). These bioinputs are considered an alternative to agrochemicals, often used excessively and inefficiently in conventional agriculture.

In the rhizosphere, plant growth promoting rhizobacteria (PGPR) stand out for their ability to colonize the rhizospheric environment. These bacteria compete effectively in this niche and perform essential functions, such as producing growth hormones, solubilizing nutrients, assimilating nitrogen, and protecting against pathogens, thereby contributing to healthy plant development (Dalolio et al., 2018). Numerous PGPRs have been isolated from the rhizosphere of various cultivated plants and studied, including *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Azotobacter*, *Staphylococcus*, and *Azospirillum* (Kang et al., 2019). These beneficial bacteria, associated with plant roots, play a crucial role in root development and the overall health of crops. Recent studies have demonstrated the potential of rhizobacteria to promote plant growth, enhance nutrient uptake, and positively influence root architecture (Fernandes et al., 2021; Cruz et al., 2022; Silva et al., 2022, 2023; Cruz et al., 2024b). These rhizobacteria can be significant in agriculture as bioinputs, particularly for economically important crops such as soybean, common bean, maize, and upland rice.

The promotion of plant growth and increased productivity by rhizobacteria can occur directly at various stages of the plant life cycle, including nitrogen fixation, phosphate solubilization, phytohormone production, enhanced mineral availability, and root morphology modification

(Swarnalakshmi et al., 2020). Phytohormone production by rhizobacteria stimulates root development by increasing cell division and elongation. Additionally, phosphate solubilization improves the availability of this nutrient, resulting in more robust roots, which can enhance nutrient uptake and resistance to abiotic stress. Alcantara et al. (2021) found that applying rhizobacteria strains led to greater root length in maize seedlings and increased grain productivity compared to the control treatment.

Bacillus species are well known for their ability to produce phytohormones, such as auxins, and enzymes that promote root development and enhance nutrient and water uptake (Timofeeva et al., 2023). A similar result was reported by Rêgo et al. (2014), where the application of rhizobacterial isolates from the genera *Burkholderia* and *Pseudomonas* in upland rice resulted in plants with greater root development and productivity compared to non-inoculated plants. Bacteria of the genus *Serratia*, on the other hand, stand out for their production of siderophores, which facilitate iron availability in the soil, and for inducing systemic resistance in plants against biotic and abiotic stresses (Cruz et al., 2024a). The interaction of these bacteria with crops promotes more robust roots and efficient root systems, enhancing initial establishment and plant performance throughout the cultivation cycle (Silva et al., 2022, 2023). These findings underscore the importance of exploring beneficial microorganisms as biotechnological tools to improve the efficiency of agricultural systems.

Despite the benefits of using beneficial microorganisms in agriculture and the growing body of research demonstrating

their efficiency in promoting initial root development, it is essential to prospect for new strains adapted to soybean, maize, common bean, and upland rice. This approach aims to expand the range of bioinputs available to enhance the development of these crops, increase productivity, and support more sustainable agricultural practices, particularly under challenging edaphoclimatic conditions. Therefore, this study aimed to determine the effects of plant growth promoting rhizobacteria on the root systems of soybean, common bean, maize, and upland rice seedlings.

Material and Methods

This study was conducted in a greenhouse at Embrapa Rice and Beans, located in Santo Antônio de Goiás, Goiás, Brazil, in March 2024. The experimental design for each crop was completely randomized, with four treatments and ten replicates. The treatments involved seed microbiolization of soybean (cultivar NS 6906), common bean (cultivar FC 402), maize (cultivar AG 8088), and upland rice (cultivar BRS A501 CL) with multifunctional microorganisms BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and a control treatment (without microorganisms), applied individually. The microorganisms were selected based on their biochemical characteristics (Table 1), identified as beneficial to crops, and stored in the microorganism collection at Embrapa Rice and Beans. Studying these microorganisms effects on the root system development of crops will complement ongoing field research.

Table 1

Collection code, geographic origin, biochemical characteristics, and taxonomic classification of the three isolates used in the present experiment

Code ^A	Origin ^B	Color ^C	Biochemical ^D					Taxonomic ^E
			IAA ^F	Celul. ^G	Phos ^H	Sider. ^I	Biofilm ^J	
BRM 32111	PA/Brazil	Yellow	-	+	+	+	+	Burkholderia sp.
BRM 63523	PA/Brazil	Red	-	+	+	+	+	Serratia sp.
BRM 63524	PA/Brazil	Yellow	+	+	+	+	-	Bacillus sp.

^ANumerical code of rhizobacteria isolates from the Embrapa Rice and Beans Multifunctional Microorganisms and Fungi collection; ^BGeographical origin of each isolate; ^CColony color, ^DBiochemical characterization and ^ETaxonomic classification of each isolate, described by Nascente et al. (2017); ^FProducer of acetic acid; ^GProducer of cellulase;

^HProducer of phosphatase; ^IProducer of siderophores; ^JProducer of biofilms.

Before microbiolization, the seeds underwent a disinfestation process involving immersion in 7.5% sodium hypochlorite for seven minutes, followed by immersion in 70% ethanol for five minutes, and three rinses in sterilized distilled water, each lasting one minute, to remove residues. The seeds were then dried at ambient temperature (29°C). Microbiolization was carried out by immersing the seeds in bacterial suspensions under constant agitation at 125 rpm, with immersion times of 4 hours for soybean (Silva et al., 2020), 2 hours for common bean (Rezende et al., 2021a), 4 hours for maize (Cruz et al., 2023), and 24 hours for upland rice (Filippi et al., 2011).

The rhizobacteria were cultured on a solid medium (nutrient agar) and subsequently suspended in liquid 523 medium (nutrient broth) (Kado & Heskett, 1970) and incubated for 24 hours at 28°C in a shaking incubator. The solution concentration was adjusted to A₅₄₀ = 0.5 (equivalent to 10⁸ CFU – Colony Forming Units) using a Hach DR 2800® spectrophotometer. 125 mL of bacterial suspension was used for every 50 g of

seeds for each crop to ensure proper seed coverage with the culture medium.

After removal from the medium and drying, the seeds were transferred to 500 mL plastic cups filled with a mixture of soil and coarse sand in a 1:1 ratio. The soil used in the mixture was classified as clay-textured latosol, collected from the arable soil layer (0.0–0.20 meters) of a slope. The soil chemical characteristics were determined according to the methods described by Claessen (1997): pH in H₂O = 5.9; organic matter (OM) = 17.6 g dm⁻³; Mehlich-P = 26.6 mg dm⁻³; K = 3 mmol_a dm⁻³; Ca²⁺ = 20 mmol_a dm⁻³; Mg²⁺ = 6 mmol_a dm⁻³; Al³⁺ = 0 mmol_a dm⁻³; H + Al = 20 mmol_a dm⁻³; SB (base saturation) = 31 mmol_a dm⁻³; CEC potential (T) = 50 mmol_a dm⁻³; effective CEC (t) = 36 mmol_a dm⁻³.

Two seeds were sown per plastic container, with no fertilization applied to the experimental units. After five days, thinning was performed, retaining only the most developed seedling. No temperature or relative humidity control was implemented in the experimental environment. Daily irrigation of the experimental units was carried out to

maintain soil moisture near field capacity, with an average water depth of 5 mm applied daily in the morning.

After 14 days of planting, the seedlings were removed by washing the soil from the containers, and the shoots were cut off for root system evaluation. Root images were captured on black ethylene vinyl acetate (EVA) material using a digital camera positioned 30 cm away, with the distance determined by a cylindrical acrylic support. The images were analyzed using WinRhizo Pro 2012® software, enabling the following measurements: total root length (LengR, cm), root diameter (DiamR, mm), total root surface area (AreaS, cm²), and root volume (VolR, cm³). These analyses provide quantitative data on plant development, health, and environmental adaptation (Böhm, 1979).

Additionally, the root system of each replicate was placed in kraft paper bags and dried in a forced ventilation oven at 65°C for 72 hours until a constant mass was achieved. After drying, root dry mass (RDM) was determined using a balance with a precision of four decimal places (0.0001).

The experiments were analyzed separately, and the collected data were subjected to analysis of variance using the Sisvar 5.6 statistical software (Ferreira et al., 2019). Means were compared using the LSD test ($p \leq 0.05$). When the correlation test indicated $r \geq 0.50$, Principal Components (PCs) were used as response variables. Biplots were constructed to correlate the isolated microorganisms and the response variables, using the Scilab statistical software to visualize the relationships between the isolated microorganisms and the analyzed variables.

Results and Discussion

Soybeans

In evaluating total root length and root surface area, the treatment with inoculation of the BRM 63524 isolate provided the best results for soybeans (Table 2), with respective increases of 35.26% and 21.28% compared to the control treatment. Similar effects were observed by Silva et al. (2022) with the application of rhizobacteria from the *Bacillus* sp. genus, which also increased root length and total surface area in soybean seedlings. According to the authors, this result may be related to the genus's ability to produce indole-3-acetic acid (IAA), a phytohormone that promotes root growth and cell elongation. This finding was supported by the determination of the biochemical characteristics of the microorganisms, which demonstrated the ability of the BRM 63524 strain to produce IAA (Table 1). Additionally, the capacity of multifunctional rhizobacteria to promote root development improves water and mineral uptake, making plants more vigorous and productive (Hungria, 2011).

No statistical differences were observed for the variables root diameter and root volume in soybean seedlings (Table 2). However, for the evaluation of root dry mass in soybean seedlings, the BRM 63523 isolate outperformed the control treatment, with an average increase of 96.68%. Silva et al. (2020) reported higher root production and dry mass in soybean plants inoculated with rhizobacteria from the *Serratia* genus, highlighting its effectiveness as a bioagent in promoting root growth. Similarly, Frasca et al. (2024), while evaluating the inoculation of *Serratia* isolates on the initial development

of soybean seedlings, also observed an increase in root dry mass, with a 14% increment compared to treatments without rhizobacteria.

Table 2

Total root length (LengR), root diameter (DiamR), total root surface area (AreaS), root volume (VolR), and root dry mass (RDM) of soybean seedlings (cultivar NS 6906) as influenced by rhizobacteria treatments. An evaluation was conducted 14 days after soybean sowing

Treatment	LengR (cm)	AreaS (cm ²)	DiamR (mm)	VolR	Phos ^H
BRM 32111 (<i>Burkholderia</i> sp.)	37.24 bc	49.99 ab	1.33 a	1.27 a	0.0240 ab
BRM 63523 (<i>Serratia</i> sp.)	42.65 ab	53.52 ab	1.56 a	1.04 a	0.0415 a
BRM 63524 (<i>Bacillus</i> sp.)	48.25 a	56.47 a	1.53 a	1.35 a	0.0297 ab
Control	35.67 c	46.56 b	1.62 a	1.02 a	0.0211 b
CV (%)	17.62	19.41	22.22	32.63	24.10

*Means followed by the same letter in the column do not differ by the LSD test ($p \leq 0.05$).

According to Araújo et al. (2021), this result can be explained by phosphorus solubilization, as enhanced nutrient absorption improves the partitioning of assimilates in soybean plants, which can lead to an increase in the root surface area of intermediate roots and root dry mass.

Common beans

The treatment with the BRM 63524 isolate showed the greatest total root length in common beans, with an increase of 41.73% compared to the control treatment, where no growth-promoting rhizobacteria were applied (Table 3). Rezende et al. (2021b) observed the ability of growth-promoting rhizobacteria to enhance root mass in common beans, attributing this effect to direct mechanisms such as phosphorus solubilization, nitrogen fixation, and/or the production of plant growth regulators like indole-3-acetic acid (IAA).

Table 3

Total root length (LengR), root diameter (DiamR), total root surface area (AreaS), root volume (VoIR), and root dry mass (RDM) of common bean seedlings (cultivar FC 402) as influenced by rhizobacteria treatments. An evaluation was conducted 14 days after common bean sowing

Treatment	LengR (cm)	AreaS (cm ²)	DiamR (mm)	VoIR (cm ³)	RDM (g)
BRM 32111 (<i>Burkholderia</i> sp.)	40.7 b	51.49 a	1.23 b	0.125 b	0.1048 a
BRM 63523 (<i>Serratia</i> sp.)	41.8 b	56.83 a	1.55 ab	0.179 ab	0.1000 a
BRM 63524 (<i>Bacillus</i> sp.)	50.6 a	53.04 a	1.85 a	0.262 a	0.0745 b
Control	35.7 b	50.30 a	1.24 b	0.119 b	0.0723 b
CV (%)	21.24	18.99	29.08	42.63	24.15

*Means followed by the same letter in the column do not differ by the LSD test ($p \leq 0.05$).

Similarly, Oliveira et al. (2016), in their study evaluating the effects of *Bacillus* genus bacteria inoculation on the initial growth of common bean, found that plants treated with these rhizobacteria exhibited increased primary root length in seedlings. These results highlight the positive effects of the *Bacillus* genus in promoting root length, significantly benefiting the common bean during its early developmental stage.

No statistical differences were observed for the variable total root surface area in common bean seedlings (Table 3). However, the BRM 63524 treatment stood out for root diameter and volume in common bean seedlings, showing increases of 49.19% and 120.16%, respectively, compared to the control treatment. According to Chagas et al. (2018), the significant increase in these parameters can be attributed to the higher production of phytohormones by the *Bacillus* genus.

The BRM 32111 and BRM 63523 isolates also outperformed the control treatment regarding root dry mass, with increases of 44.95% and 38.31%, respectively. According to Zucareli et al.

(2018), the greater root mass accumulation may be linked to the microorganisms ability to produce growth-promoting phytohormones such as auxins and gibberellins, as well as pectinase, and to enhance phosphorus availability in the soil through solubilization.

Maize

The treatments BRM 63523, BRM 32111, and BRM 63524 significantly increased the total root length of maize compared to the control treatment, with increments of 38.09%, 36.50%, and 25.39%, respectively (Table 4). Similar results were reported by Cruz et al. (2024b), who found that the application of *Serratia* sp. and *Bacillus* sp. promoted greater elongation and, consequently, greater total root length in maize seedlings compared to the control treatment. Likewise, Cruz et al. (2022) observed that maize seedlings inoculated with isolates of *Serratia* sp., *Bacillus* sp., and the isolate BRM 32111 exhibited statistically superior total root length compared to non-inoculated plants.

Table 4

Total root length (LengR), root diameter (DiamR), total root surface area (AreaS), root volume (VolR), and root dry mass (RDM) of maize seedlings (cultivar AG 8088) as influenced by rhizobacteria treatments. An evaluation was conducted 14 days after maize sowing

Treatment	LengR (cm)	AreaS (cm ²)	DiamR (mm)	VolR (cm ³)	RDM (g)
BRM 32111 (<i>Burkholderia</i> sp.)	86 a	27.90 a	0.77 b	0.193 ab	0.1490 a
BRM 63523 (<i>Serratia</i> sp.)	87 a	29.38 a	0.79 b	0.219 a	0.1366 ab
BRM 63524 (<i>Bacillus</i> sp.)	79 a	28.46 a	0.84 a	0.234 a	0.1275 ab
Control	63 b	27.29 a	0.76 b	0.180 b	0.1265 b
CV (%)	6.33	7.57	5.61	20.13	14.61

*Means followed by the same letter in the column do not differ by the LSD test ($p \leq 0.05$).

No significant differences were observed for the total root surface area of maize seedlings (Table 4). However, seedlings treated with BRM 63524 exhibited a higher average root diameter than the other treatments. This treatment was the only one to show a significant increase in root diameter compared to the control, with an increment of 10.52%. Milani et al. (2019) highlight the relevance of the *Bacillus* sp. genus among various PGPR genera, emphasizing its successful use over several decades. This genus has demonstrated significant effects on plant growth promotion, directly influencing root morphology due to its capabilities, such as auxin production, nitrogen fixation, phosphorus solubilization, and antifungal activity.

Regarding root volume in maize seedlings, the isolates BRM 63524 and BRM 63523 stood out statistically compared to the control treatment, with increases of 30% and 21.67%, respectively. In the evaluation of root dry mass, the treatment with BRM 32111 was noteworthy, showing an increase of 17.79% compared to the control. The greater root elongation promoted by phytohormones

produced by the *Burkholderia* genus, coupled with the expansion of the soil exploration zone, naturally enhances nutrient absorption and accumulation in root tissues, leading to an increase in maize root dry mass (Cruz et al., 2024a).

Upland rice

The treatment with BRM 63524 resulted in the greatest total root length in upland rice seedlings, being the only treatment statistically different from the control (Table 5). This isolate showed an increase of 93.22% compared to the absence of multifunctional rhizobacteria application. Consistent with these findings, Silva et al. (2023) reported that using *Bacillus* genus bacteria promoted greater total root length in upland rice seedlings, contrasting with previously reported data. The authors attributed this result directly to the auxin production capability of the genus. Conversely, Nascente et al. (2023) associated the increase in root diameter of upland rice seedlings with enhanced physiological processes in treated plants compared to untreated ones.

Table 5

Total root length (LengR), root diameter (DiamR), total root surface area (AreaS), root volume (VoIR), and root dry mass (RDM) of upland rice seedlings (cultivar BRS A501 CL) as influenced by rhizobacteria treatments. An evaluation was conducted 14 days after upland rice sowing

Treatment	LengR (cm)	AreaS (cm ²)	DiamR (mm)	VoIR (cm ³)	RDM (g)
BRM 32111 (<i>Burkholderia</i> sp.)	75 b	18.63 a	0.65 a	0.131 a	0.0264 a
BRM 63523 (<i>Serratia</i> sp.)	73 b	19.93 a	0.64 a	0.130 a	0.0224 a
BRM 63524 (<i>Bacillus</i> sp.)	114 a	19.76 a	0.68 a	0.133 a	0.0263 a
Control	59 b	19.52 a	0.66 a	0.127 a	0.0187 b
CV (%)	17.1	17.3	16.4	28.9	33.93

*Means followed by the same letter in the column do not differ by the LSD test ($p \leq 0.05$).

Sousa et al. (2019) observed that applying *Bacillus* isolates led to an average increase of 24.3% in root length in flooded irrigated rice seedlings of the BRS Catiana cultivar. No significant differences were observed for total root surface area, diameter, or volume in upland rice seedlings. Thus, the ability of the BRM 63524 strain to produce IAA (Table 1) likely contributed to the greater root development observed in rice seedlings in the present experiment.

The efficiency of inoculating upland rice seedlings was observed with the application of the isolates BRM 32111, BRM 63524, and BRM 63523, resulting in increases in root dry mass of 41.18%, 40.64%, and 19.79%, respectively, compared to the control treatment (Table 5). Silva et al. (2023) found that the application of *Bacillus* and *Serratia* isolates was capable of promoting greater biomass accumulation in both roots and shoots of upland rice seedlings, whether applied individually or as co-inoculations, compared to the absence of rhizobacteria. In their study on upland rice seedlings, Sousa et

al. (2019) observed that inoculation with the BRM 32111 isolates provided the greatest root growth and, consequently, the highest biomass accumulation compared to non-inoculated seedlings.

Principal component analysis

Principal component analysis (PCA) was performed to reduce data dimensionality, identify significant patterns, and highlight the most relevant variables, facilitating the interpretation and visualization of results. Regarding the PCA for the experiments with soybean, common bean, maize, and upland rice seedlings, it was observed that the variability of treatments with isolated microorganisms concerning total root length (RootL), root diameter (RootD), total root surface area (RootSA), root volume (RootV), and root dry mass (RDM) in seedlings treated with multifunctional microorganisms was best explained by two principal components (PC1 and PC2; Figure 1).

The factor maps (biplots) illustrate groups of variables (arrows) indicating positive and negative correlations with each principal component, with arrow length representing the magnitude of each response for each PC (Figures 1B, 1D, 1F, and 1H). The microorganism BRM 63524 positively correlated with the measured parameters across all crops. Inoculation with this isolate positively correlated with total root length in soybean, common bean, and upland rice. A positive correlation was also observed for root diameter and volume in common bean, maize, and upland rice. Furthermore, BRM 63524 positively correlated with the total root surface area in soybeans.

The microorganism BRM 32111 only positively correlated with root dry mass in common bean, maize, and rice crops. The isolate BRM 63523 positively correlated with the total root surface area in common beans and maize. Additionally, it was associated with root diameter and root dry mass in soybeans, as well as root length in maize. The control treatment did not correlate positively with the evaluated crop variables.

Greater root development was observed in the root system images of soybean (Figures 2A–2D), common bean (Figures 2E–2H), maize (Figures 2I–2L),

and upland rice (Figures 2M–2P) seedlings treated with multifunctional microorganisms compared to the control treatment. Thus, inoculation with RPCV isolates demonstrated beneficial effects on grain crop cultivation. This is particularly significant as more vigorous root growth enhances the plant's ability to absorb nutrients and water from the soil while increasing resilience to adverse conditions such as drought, pests, and diseases.

As Shoaib et al. (2022) stated, root system plasticity is a key factor in adaptation to adverse conditions, providing greater efficiency in water and nutrient use. Consequently, root architecture adaptation is essential to tolerate biotic and abiotic stresses across various crops. Furthermore, this contributes to stronger plant anchorage and stability in the soil, ultimately improving overall productivity. For instance, Fernandes et al. (2020) reported that upland rice plants treated with multifunctional rhizobacteria exhibited greater root development, leading to higher grain yields. Similarly, Nascente et al. (2017) highlighted that more vigorous early development can positively impact several physiological aspects, such as nutrient uptake and plant vigor, with the potential to increase crop productivity.

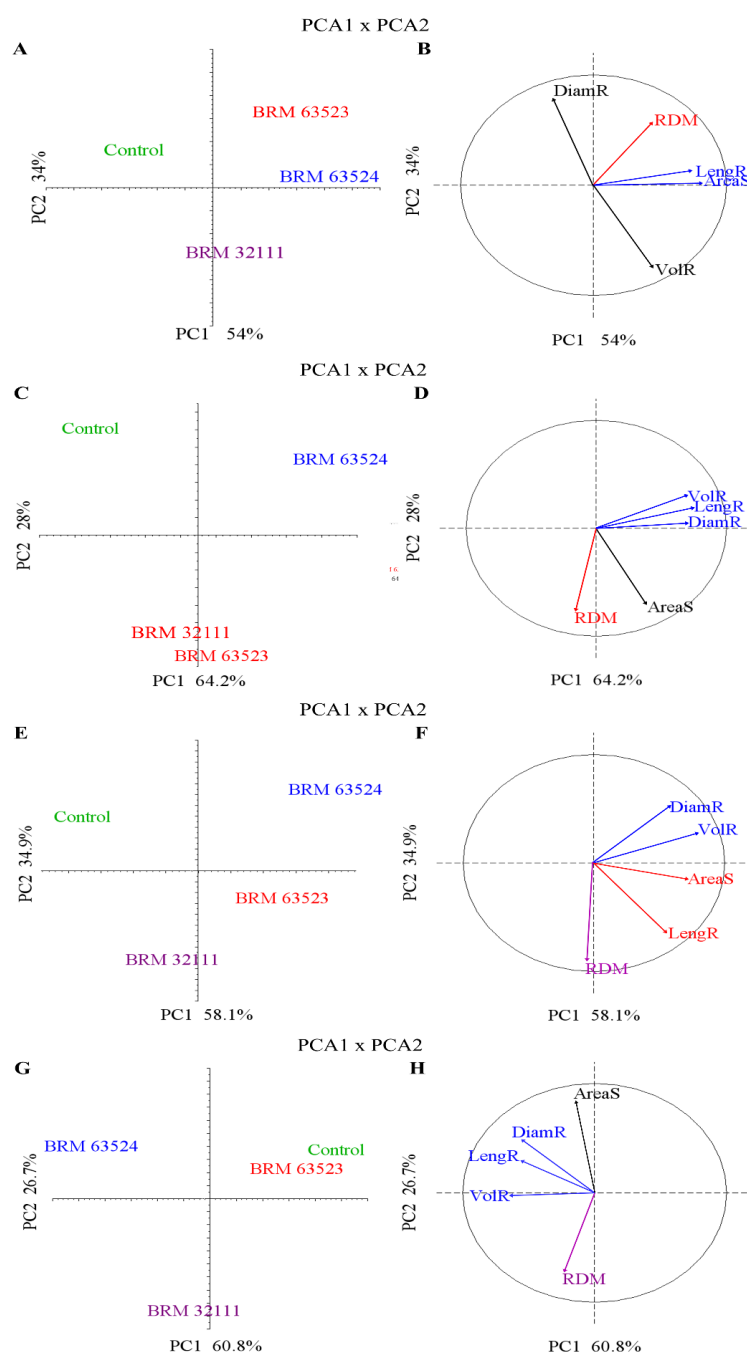


Figure 1. Principal Component Analysis (PCA) PCA1 vs. PCA2, explaining the correlations between the evaluated variables and the four treatments with isolated multifunctional microorganisms and the control (no microorganisms) inoculated in soybean (A and B), common bean (C and D), maize (E and F), and upland rice seedlings (G and H).

A, C, E, and G: Biplot for treatments BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control treatment (no microorganisms). B, D, F, and H: Correlation circle plot of the variables. Color schemes (red, green, blue, purple, and black [no correlation]) were used to enhance the visualization of correlations between the variable and treatment correlation plots within each crop, with matching colors indicating correlations.

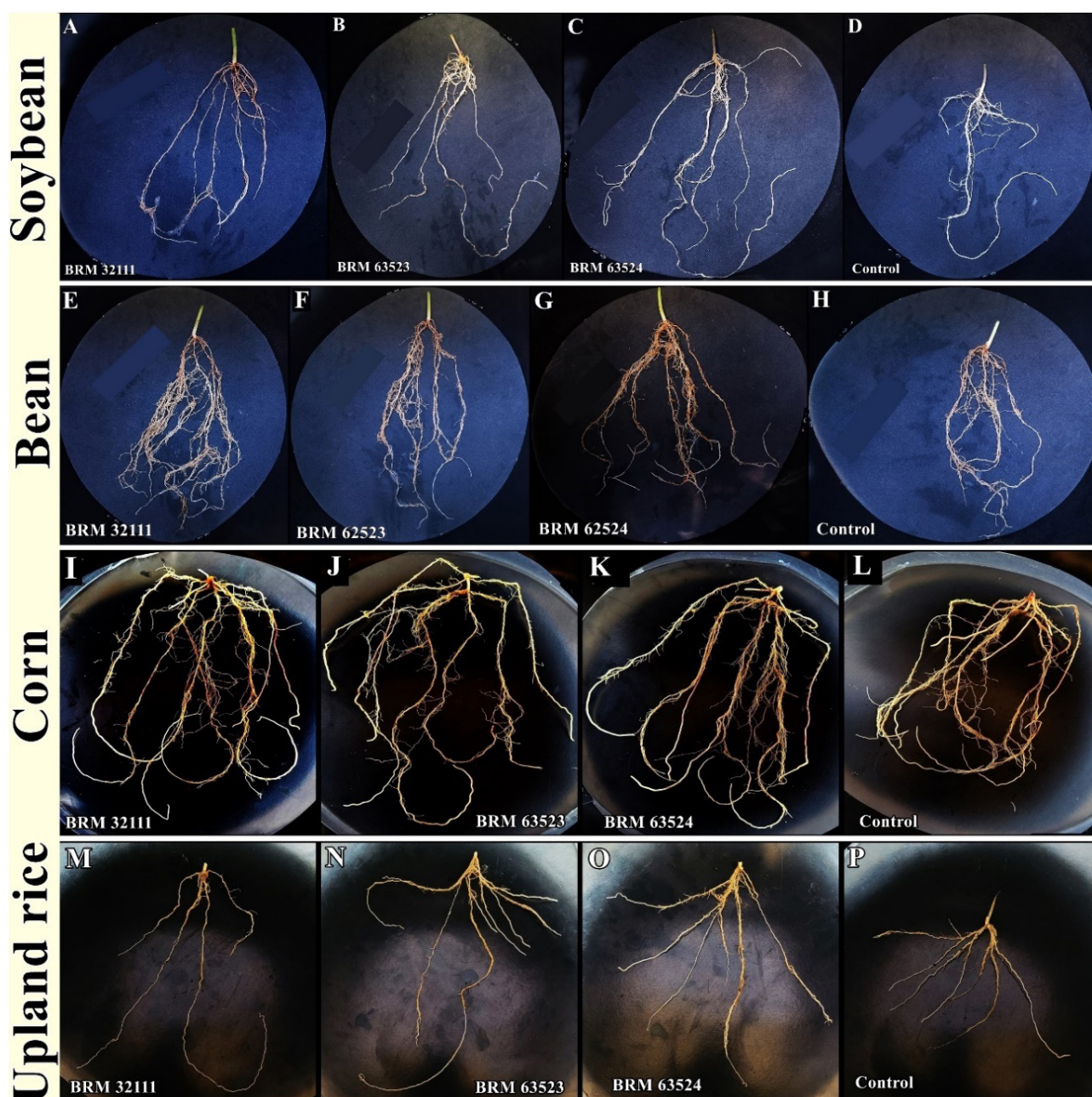


Figure 2. Root systems of seedlings subjected to seed microbiolization with multifunctional rhizobacteria. Soybean seedlings:

A. BRM 32111 (*Burkholderia* sp.), B. BRM 63523 (*Serratia* sp.), C. BRM 63524 (*Bacillus* sp.), and D. control treatment (no microorganisms). Common bean seedlings: E. BRM 32111, F. BRM 63523, G. BRM 63524, and H. control treatment. Maize seedlings: I. BRM 32111, J. BRM 63523, K. BRM 63524, and L. control treatment. Upland rice seedlings: M. BRM 32111, N. BRM 63523, O. BRM 63524, and P. control treatment. The seedlings were photographed 14 days after sowing using a digital camera.

Conclusions

Using rhizobacteria has induced positive changes in the root architecture of soybean, common bean, maize, and upland rice seedlings. The isolate BRM 63524 showed the most significant effects in promoting root growth across the evaluated crops, particularly in total root length. Seedlings not inoculated with rhizobacteria tend to exhibit inferior root development compared to inoculated ones. These results demonstrate that rhizobacteria can promote root growth in the evaluated crops, potentially sustainably enhancing productivity.

References

- Alcantara, R. M. C. M., Sousa, T. S., Peres, T. L., Oliveira, A. K. S., Silva, E. A., & Costa, P. M. (2021). *Parâmetros morfológicos e produtivos da cultura do milho inoculado com bactérias promotoras de crescimento vegetal em Teresina-PI*. Boletim de Pesquisa e Desenvolvimento da EMBRAPA Meio-Norte.
- Amaral, D. F. S., Lira, V. M. C., Mazzaro, M. A. T., Nogueira, J. D., & Vidal, M. C. (2021). Bioinsumos: a construção de um programa nacional pela sustentabilidade do agro brasileiro. *Economic Analysis of Law Review*, 12(3), 557-574.
- Araújo, F. F., Bonifácio, A., Bavaresco, L. G., Mendes, L. W., & Araújo, A. S. F. (2021). *Bacillus subtilis* changes the root architecture of soybean grown on nutrient-poor substrate. *Rhizosphere*, 18(2021), e100348. doi: 10.1016/j.rhisph.2021.100348
- Böhm, W. (1979). *Methods of studying root systems*. Springer.
- Chagas, L. F. B., Martins, A. L. L., Carvalho, M. R., Fº., Miller, L. de O., Oliveira, J. C., & Chagas, A. F., Jr. (2018). *Bacillus subtilis* e *Trichoderma* sp. no incremento da biomassa em plantas de soja, feijão-caupi, milho e arroz. *Agri-Environmental Sciences*, 3(2), 10-18. doi: 10.36725/agries.v3i2.430
- Claessen, M. E. C. (1997). *Manual for methods of soil analysis* (2ª ed.). Embrapa Solos.
- Cruz, D. R. C., Ferreira, I. V. L., Monteiro, N. O. da C., Nascente, A. S., Oliveira, R. B., Santos, S. G. F. dos, Rosa, C. O., & Vieira, I. C. de O. (2024a). Microorganismos multifuncionais na agricultura: uma revisão sistemática sobre bactérias solubilizadoras de fósforo. *Contribuciones A Las Ciencias Sociales*, 17(3), e5854. doi: 10.55905/revconv.17n.3-358
- Cruz, D. R. C., Nascente, A. S., Silva, M. A., & Barroso, J., Neto. (2022). Root and shoot development of corn seedlings as affected by rhizobacteria. *Colloquium Agrariae*, 18(1), 53-63. doi: 10.5747/ca.2022.v18.n1.a479
- Cruz, D. R. C., Silva, M. A., Ferreira, I. V. L., & Ribeiro, A. E. C. (2024b). Effects of isolated and combined growth-promoting microorganisms altering root and shoot development of corn seedlings. *Amazonian Journal of Agricultural and Environmental Sciences*, 67(1), 1-13. doi: 10.5281/zenodo.10979214358
- Cruz, D. R. C., Silva, M. A., Nascente, A. S., Filippi, M. C. C., & Ferreira, E. P. B. (2023). Use of multifunctional microorganisms in

- corn crop. *Revista Caatinga*, 36(2), 349-361. doi: 10.5747/ca.2022.v18.n1.a479
- Dalolio, R. S., Borin, E., Cruz, R. M. S., & Alberton, O. (2018). Co-inoculação de soja com *Bradyrhizobium* e *Azospirillum*. *Journal of Agronomic Sciences*, 7(2), 1-7.
- Fernandes, J. P. T., Nascente, A. S., Filippi, M. C. C. de, Lanna, A. C., Sousa, V. S., & Silva, M. A. (2020). Physio-agronomic characterization of upland rice inoculated with mix of multifunctional microorganisms. *Revista Caatinga*, 33(3), 679-689. doi: 10.1590/1983-21252020v33n311rc
- Fernandes, J. P. T., Nascente, A. S., Filippi, M. C. C., & Silva, M. A. (2021). Upland rice seedling performance promoted by multifunctional microorganisms. *Semina: Ciências Agrárias*, 42(1), 429-438. doi: 10.5433/1679-0359.2021v42n1p429
- Ferreira, D. F. (2019). Sisvar: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, 37(4), 529-535. doi: 10.28951/rbb.v37i4.450
- Filippi, M. C. C., Silva, G. I. B., Silva-Lobo, V. L., Côrtes, M. V. C. B., Moraes, A. J. G., & Prabhu, A. S. (2011). Leaf blast (*Magnaporthe oryzae*) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil. *Biological Control*, 58(2), 160-166. doi: 10.1016/j.biocontrol.2011.04.016
- Frasca, L. L. M., Rezende, C. C., Silva, M. A., Lanna, A. C., Cruz, D. R. C., & Nascente, A. S. (2024). Interação de plântulas de soja com rizobactérias benéficas. *Semina: Ciências Agrárias*, 44(6), 2217-2228. doi: 10.5433/1679-0359.2023v44n6p2217
- Hungria, M. (2011). *Inoculação com Azospirillum brasiliense: Inovação em rendimento a baixo custo*. EMBRAPA Soja.
- Instituto Chico Mendes de Conservação da Biodiversidade (2024). *Biodiversidade do cerrado*. ICMBio. <https://www.icmbio.gov.br/cbc/conservacao-da-biodiversidade/biodiversidade.html>
- Kang, S. M., Khan, A. L., Waqas, M., Asaf, S., Lee, K. E., Park, Y. G., Kim, A. Y., Khan, M. A., You, Y. H., & Lee, I. J. (2019). Integrated phytohormone production by the plant growth-promoting rhizobacterium *Bacillus tequilensis* SSB07 induced thermotolerance in soybean. *Journal of Plant Interactions*, 14(1), 416-423. doi: 10.1080/17429145.2019.1640294
- Kado, C. I., & Heskett, M. G. (1970). Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology*, 60(6), 969-976. doi: 10.1094/phyto-60-969
- Milani, R., Santos, R. M., Bentes, L. L., Kandasamy, S., Lazarovits, G., & Rigobelo, E. C. (2019). *Bacillus subtilis* isolates with different abilities to promote plant growth in corn, cotton, and soybean crops. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences*, 21(4), 827-836.
- Nascente, A. S., Filippi, M. C., Lanna, A. C., Souza, V. L., Silva, L., & Silva, G. B. (2017). Biomass, gas exchange, and nutrient contents in upland rice plants affected by application forms of microorganism growth promoters. *Environmental Science and Pollution Research*, 24, 2956-2965. doi: 10.1007/s11356-016-8013-2

- Nascente, A. S., Ishola, Z. T., Filippi, M. C. C., & Cruz, D. R. C. (2023). Beneficial microorganisms as affecting root development of upland rice. *African Journal of Microbiology Research*, 17(1), 184-192. doi: 10.5897/AJMR2023.9695
- Ogino, C. M., Gasques, J. G., & Vieira, J. E. R., Fº. (2023). *Relação dinâmica: fertilizantes minerais e agricultura brasileira*. IPEA.
- Oliveira, G. R. F., Silva, M. S., Marciano, T. Y. F., Proença, S. L., & Sá, M. E. (2016). Crescimento inicial do feijoeiro em função do vigor de sementes e inoculação com *Bacillus subtilis*. *Revista Brasileira de Engenharia de Biosistemas*, 10(4), 439-448. doi: 10.18011/bioeng2016v10n4p439-448
- Rêgo, M. C. F., Ilkiu-Borges, F., Filippi, M. C. C., Gonçalves, L. A., & Silva, G. B. (2014). Morphoanatomical and biochemical changes in the roots of rice plants induced by plant growth-promoting microorganisms. *Journal of Botany*, 2014(818797), 1-10. doi: 10.1155/2014/818797
- Rezende, C. C., Frasca, L. L. M., Silva, M. A., Pires, R. A. C., Lanna, A. C., Filippi, M. C. C., & Nascente, A. S. (2021b). Physiological and agronomic characteristics of the common bean as affected by multifunctional microorganisms. *Semina: Ciências Agrárias*, 42(2), 599-618. doi: 10.5433/1679-0359.2021v42n2p599
- Rezende, C. C., Nascente, A. S., Silva, M. A., Frasca, L. L. M., Pires, R. A. C., Filippi, M. C. C., Lanna, A. C., & Silva, J. F. A. (2021a). Physiological and agronomic performance of common bean treated with multifunctional microorganisms. *Revista Brasileira de Ciências Agrárias*, 16(4), 1-9. doi: 10.5433/1679-0359.2021v42n2p599
- Shoaib, M., Banerjee, B. P., Hayden, M., & Kant, S. (2022). Roots' drought adaptive traits in crop improvement. *Plants*, 11(17), 2256. doi: 10.3390/plants11172256
- Silva, M. A., Nascente, A. S., Cruz, D. R. C., Frasca, L. L. M., Silva, J. F. A., Ferreira, A. L., Ferreira, E. P. B., Lanna, A. C., Bezerra, G. A., & Filippi, M. C. C. (2023). Desenvolvimento inicial de arroz de terras altas inoculado e coinoculado com rizobactérias multifuncionais. *Semina: Ciências Agrárias*, 44(1), 273-284. doi: 10.5433/1679-0359.2023v44n1p273
- Silva, M. A., Nascente, A. S., Filippi, M. C. C., Lanna, A. C., Silva, G. B., & Silva, J. F. A. (2020). Individual and combined growth-promoting microorganisms affect biomass production, gas exchange and nutrient content in soybean plants. *Revista Caatinga*, 33(3), 619-632. doi: 10.1590/1983-21252020v33n305rc
- Silva, M. A., Cruz, D. R. C., Frasca, L. L. M., Filippi, M. C. C., Ferreira, A. L., & Nascente, A. S. (2022). Inoculation and co-inoculation with multifunctional rhizobacteria for the initial development of soybean. *Pesquisa Agropecuária Tropical*, 52(1), 1-7. doi: 10.1590/1983-40632022v5273558
- Sousa, I. M., Nascente, A. S., & Filippi, M. C. C. (2019). Bactérias promotoras do crescimento radicular em plântulas de dois cultivares de arroz irrigado por inundação. *Colloquium Agrariae*, 15(2), 140-145. doi: 10.5747/ca.2019.v15.n1.a293

- Swarnalakshmi, K., Yadav, V., Tyagi, D., Dhar, D. W., Kannepalli, A., & Kumar, S. (2020). Significance of plant growth promoting rhizobacteria in grain legumes: Growth promotion and crop production. *Plants*, 9(11), e1596. doi: 10.3390/plants9111596
- Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2023). Plant growth-promoting bacteria of soil: designing of consortia beneficial for crop production. *Microorganisms*, 11(12), e2864. doi: 10.3390/microorganisms11122864
- Zucareli, C., Barzan, R. R., Silva, J. B., & Chaves, D. P. (2018). Phosphate association and inoculation with *Bacillus subtilis* and its effect on bean growth and productive performance. *Revista Ceres*, 65(2), 1793-1802. doi: 10.1590/0034-737X201865020011

