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In vitro root growth of rice seedlings inoculated with rhizobacteria

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ABSTRACT: Multifunctional microorganisms can play a crucial role in the development of the plant root system, a characteristic that significantly affects agricultural productivity. This study aimed to determine the effect of inoculating isolates of multifunctional rhizobacteria on the in vitro root growth of upland rice seedlings. The experiment had a completely randomized design with four treatments and ten replications. The treatments involved inoculating upland rice with the following rhizobacteria: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and the control treatment (without microorganisms). Inoculation with the rhizobacteria BRM 32111 and BRM 63524 had a positive effect on the initial root development of upland rice. Inoculation with isolate BRM 32111 generated significant effects on the length, diameter, total surface area and volume of rice seedling roots. The isolate BRM 63524 had significant effects on the length, diameter and total surface area of the roots. Rice seedlings from the control treatment showed the worst results for the variables analyzed. The use of PGPRs as bioagents is a promising alternative for better root development of rice seedlings.

Key words: *Bacillus* sp.; *Burkholderia* sp.; *Oryza sativa* L.; *Serratia* sp.; sustainability

Crescimento radicular *in vitro* de plântulas de arroz inoculadas com rizobactérias

RESUMO: Os microrganismos multifuncionais podem desempenhar papel crucial no desenvolvimento do sistema radicular de plantas, característica que afeta significativamente a produtividade agrícola. Este estudo teve como objetivo determinar o efeito da inoculação de isolados de rizobactérias multifuncionais no crescimento radicular in vitro de plântulas de arroz de terras altas. O experimento teve delineamento inteiramente casualizado com 4 tratamentos e dez repetições. Os tratamentos envolveram a inoculação do arroz de terras com as rizobactérias: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.) e o tratamento controle (sem microrganismos). A inoculação com as rizobactérias BRM 32111 e BRM 63524 proporcionou efeitos positivos no desenvolvimento radicular inicial do arroz de terras altas. A inoculação com o isolado BRM 32111 gerou efeitos significativos no comprimento, diâmetro, área de superfície total e volume das raízes de plântulas de arroz. O isolado BRM 63524 proporcionou efeitos significativos no comprimento, diâmetro e área de superfície total das raízes. Plântulas de arroz do tratamento controle apresentaram os piores resultados para as variáveis analisadas. O uso de RPCPs como bioagentes é uma alternativa promissora para melhor desenvolvimento radicular de plântulas de arroz.

Palavras-chave: *Bacillus* sp.; *Burkholderia* sp.; *Oryza sativa* L.; *Serratia* sp.; sustentabilidade

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Introduction

Rice (*Oryza sativa* L.) is currently the third largest cereal produced on the planet, behind only corn and wheat, with world production estimated at 778 million tons in 2022 (FAO, 2023). The crop can be grown in two ecosystems: lowland in humid floodplains with or without irrigation and highland, with supplementary irrigation or dependent on rainwater (rainfed). However, due to the reduction in the availability of water for irrigation due to the increase in human and industrial consumption, rice cultivation in the upland environment has been growing in importance worldwide (Santos et al., 2015). In Brazil, most upland rice plantations are located in the Cerrado biome, especially in the Central-West region, and are predominantly rainfed (81% of the area), rather than irrigated (Coelho, 2021).

Within this context, in the 1980s, upland rice had a large production area, but major challenges such as inadequate or irregular soil moisture supply, competition with weeds, water deficit, lack of cultivars tolerant to biotic and abiotic stresses, nutritional imbalance, inadequate cultural practices and inefficient pest and disease control led to a drastic reduction in the area under cultivation (Namuco et al., 2009). Raising the productivity of upland rice has become a significant challenge due to both biotic and abiotic stresses. This includes the incidence of diseases, long periods of drought, nutritional deficiencies, along with issues related to the initial establishment and growth of plants in no-till areas (Filippi et al., 2011).

The rhizosphere is the epicenter of interactions between plant roots, soil, microorganisms and the environment. It is an environment abundantly rich in nutrients and is home to a vast diversity of bacteria and fungi (Nascente et al., 2023). Plant development can be influenced neutrally, favorably or unfavorably by each of these elements. Several plant growth promoting rhizobacteria (PGPRs) have been frequently isolated from the rhizosphere of various cultivated plants and have been studied, such as: *Agrobacterium, Arthrobacter, Bacillus, Burkholderia, Pseudomonas, Serratia, Azotobacter, Staphylococcus*, and *Azospirillum* (Mahmood et al., 2016).

In this scenario, the use of multifunctional microorganisms in upland rice cultivation systems is emerging as a management strategy. Its purpose is to boost plant growth by expanding the development of the root system, increasing nutrient absorption and optimizing gas exchange (Fernandes et al., 2021). This process consequently results in an increase in plant biomass and grain productivity (Nascente et al., 2017). The ability of this group of microorganisms to promote plant growth is closely linked to their positive influence on the physiological characteristics of development.

These microorganisms employ a variety of mechanisms of action, such as solubilizing nutrients, inhibiting the growth of pests and pathogens, producing bactericidal and antifungal substances, growth hormones, siderophores, and biological nitrogen fixation (Lakshmanan et al., 2015). The main benefit of these mechanisms is the more robust development of the root system, allowing the plant to explore a larger area of soil and increase the absorption of water and nutrients (Cruz et al., 2022). Several authors have described the use of plant growth promoting rhizobacteria in upland rice as beneficial, describing their ability to promote better initial root development in seedlings (Fernandes et al., 2021; Nascente et al., 2023; Silva et al., 2023).

It is therefore interesting to identify a greater number of microorganisms that positively affect the initial root development of rice plants. Therefore, the objective of this study was to determine how the inoculation of multifunctional rhizobacteria isolates affects the *in vitro* root growth of upland rice seedlings.

Materials and Methods

The research was conducted at the Laboratório de Microbiologia Agrícola of Embrapa Arroz e Feijão, located in Santo Antônio de Goiás, Goiás, Brazil, during the month of September 2022. The experimental design was completely randomized with four treatments and ten replicates. The treatments consisted of microbiolizing the seeds (a process that puts the seeds in contact with the solution of microorganisms to be tested) of the upland rice cultivar BRS A501 CL with the multifunctional microorganisms BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and the control treatment (without microorganisms), inoculated in isolation. The multifunctional microorganisms used in this experiment were selected from the collection of agriculturally relevant microorganisms maintained by Embrapa Arroz e Feijão.

Before microbiolization, the seeds were subjected to a disinfestation process. This included immersion in 7.5% sodium hypochlorite for seven minutes, followed by treatment with 70% alcohol for five minutes, with three washes in distilled water of one minute each to remove residues. The seeds were then dried at a room temperature of 29 °C. The microbiolization of the rice seeds followed the methodology described by Filippi et al. (2011), which consists of immersing the seeds in bacterial suspensions for a period of 24 hours. The rhizobacteria were grown on solid medium (nutrient agar) and then suspended in liquid medium 523 (nutrient broth) (Kado & Heskett, 1970) and incubated for 24 hours at 28 °C in a shaking incubator. After this period, the concentration of the solution was adjusted to A540 = 0.5 (equivalent to 10^8 CFU - Colony Forming Units) using spectrophotometry.

After drying, the seeds were transferred to the test tubes using the tip of a spatula. Each experimental unit consisted of a grain of upland rice (cultivar BRS A501 CL), arranged for germination in 150 mL test tubes containing agar medium (0.8 m/v) enriched with N-P-K in a total volume of 40 mL, according to the methodology of Nascente et al. (2023), adapted from Oliveira et al. (2022). The tubes were then placed in a germination room at 28 °C with a 12 hours light cycle and removed 15 days after sowing.

After removing the seedlings from the agar medium and washing their roots to remove excess soil, the root system was separated from the aerial part. Then, images of the roots were captured on a black cloth using a digital camera. The images obtained were analyzed using WinRhizo Pro 2012® software, which made it possible to take the following measurements: total root length (LengR, cm), root diameter (DiamR, mm), total root surface area (AreaS, cm^2), and root volume (VolR, cm^3). These analyses can offer important insights into the development, health and adaptation of plants to the environment, providing quantitative data on their roots.

The data collected was subjected to analysis of variance using Sisvar 5.6 statistical software (Ferreira, 2019), and the means were compared using the LSD test ($p \leq 0.05$). When the result of the correlation test indicated $r \geq 0.50$, the Principal Components (PCs) were used as response variables. The first two PCs explained 98.9% of the variation in the data. A biplot (two-dimensional graph) was generated to correlate the isolated microorganisms and the response variables, using Scilab statistical software. This biplot made it possible to visualize the relationships between the isolated microorganisms and the variables analyzed.

Results and Discussion

The root length (ranged from 12.96 to 16.55 cm), diameter (ranged from 0.58 to 0.86 mm), surface area (ranged from 10.50 to 16.66 cm^2), and volume (ranged from 0.076 to 0.143 cm^3) of upland rice seedlings were affected by the microorganisms and differed from the control (without microorganisms) (Figures 1 to 4). In the evaluation of total root length, isolates BRM 32111 and BRM 63524 were statistically superior, with respective increases of 27.70 and 25.23% compared to the control treatment (Figure 1). The considerable increase in root length may be related to the fact that the genera *Burkholderia* sp. and *Bacillus* sp. are recognized for their ability to produce growth phytohormones, especially auxins (Silva et al., 2020), and to increase the availability of nutrients (Nascente et al., 2023). By producing indole acetic acid (IAA), rhizobacteria can promote root elongation, resulting in greater length, which allows plants to more efficiently exploit the resources available in the soil (Guo et al., 2021).

The application of isolates BRM 32111 and BRM 63524 stimulated root elongation, leading to greater length, which enables plants to exploit the resources present in the soil more widely. Silva et al. (2023) found that the use of bacteria from the *Bacillus* genus promoted greater total root length in upland rice seedlings, as in the current study, a fact that the authors relate directly to the genus ability to produce auxins. Maqsood et al. (2020) working with *Burkholderia* sp. inoculation in rice plants, recorded a 27.53% increase in root length in inoculated plants compared to non-inoculated control plants. The application of PGPRs can significantly influence the morphology of plant roots, especially by promoting an increase in their length (Dar et al., 2018). This change can result in the plants being better able to exploit the soil and absorb nutrients.

The upland rice seedlings treated with isolates BRM 32111 and BRM 63524 had the largest root diameters (Figure 2). The increases in diameter compared to the control treatment were 48.27% for isolate BRM 63524 and 44.83% for isolate BRM 32111. Fernandes et al. (2021) also found greater average root diameters of upland rice seedlings treated with isolates of the genus *Bacillus* sp. Sousa et al. (2019) also observed that the use of *Bacillus* sp. isolates provided significant changes in the root architecture of irrigated rice plants. In a study conducted by Rêgo et al. (2014), anatomical and biochemical changes in the roots of upland rice plants were analyzed after treatment with isolate BRM 32111. The researchers concluded that this isolate caused significant changes in root structure, including

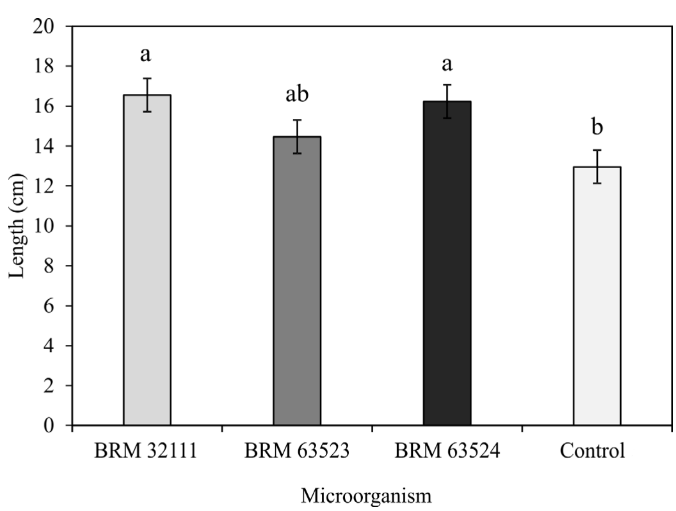

Figure 1. Total root length (LengR) of rice cultivar BRS 501 CL, as a function of treatment with rhizobacteria: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control (without microorganisms). Evaluation carried out 15 days after sowing in the test tubes.

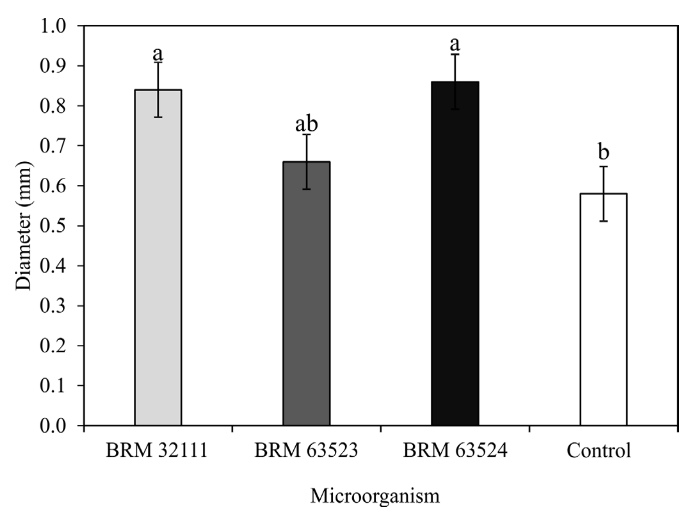

Figure 2. Root diameter (DiamR) of rice seedlings cultivar BRS 501 CL, as a function of treatment with rhizobacteria: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control (without microorganisms). Evaluation carried out 15 days after sowing in the test tubes.

an increase in root length and diameter, as well as expansion of the cortex and aerenchyma spaces.

In terms of total root surface area, the treatments with the rhizobacterial isolates were statistically superior to the control treatment (Figure 3). The treatment with isolate BRM 32111 showed the greatest increase compared to the control treatment, with a 39.62% greater surface area, followed by isolate BRM 63524 with 35.02% and finally isolate BRM 63523 with 23.62%. Cruz et al. (2022), evaluating the root development of corn seedlings treated with growth promoting rhizobacteria, found that the application of *Bacillus* sp. and *Serratia* sp. promoted greater root surface area, which is a significant indicator in understanding soil-plant interaction, as it represents the extent of the root surface available for exploration. Microorganisms capable of increasing this area tend to help improve plants ability to capture nutrients, water and other resources from the soil. According to Sperandio et al. (2017), the use of PGPRs can provide a greater amount of surface area and root biomass in upland rice seedlings, which has a direct impact on aerial part biomass and crop productivity.

In the evaluation of root volume, the BRM 32111 treatment stood out as superior to the other treatments, with an increase of 88.15% compared to the control treatment (Figure 4). According to Ahemad & Kibret (2014), an increase in the total volume of roots makes it easier for plants to absorb water and nutrients, mainly due to the increase in root development. This results in greater access to and use of these resources, as pointed out by Glick (2012). Guimarães & Baldani (2013), working with the application of *Burkholderia* sp. to corn, concluded that the increase in the accumulation of dry matter in the aerial part and root, together with grain yield in the experiment, is due to the effects caused by the presence of the rhizobacterium, which may be diverse beyond simple biological nitrogen fixation, including possible hormonal influences that stimulate an increase in root volume.

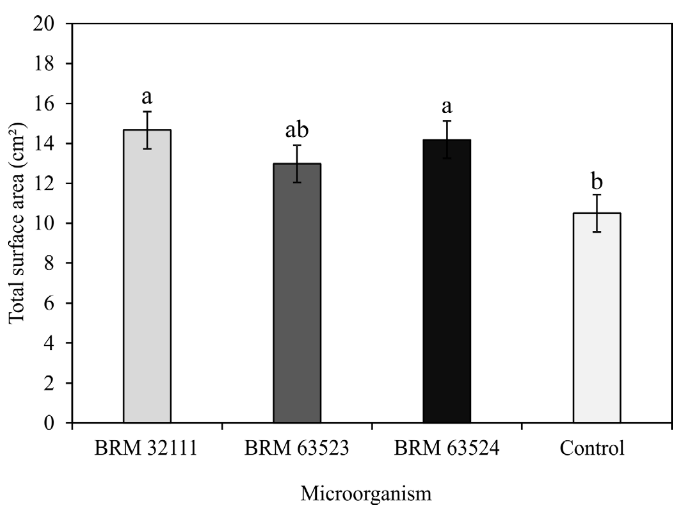

Figure 3. Total surface area of roots (AreaS) of rice seedlings cultivar BRS 501 CL, as a function of treatment with rhizobacteria: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control (without microorganisms). Evaluation carried out 15 days after sowing in the test tubes.

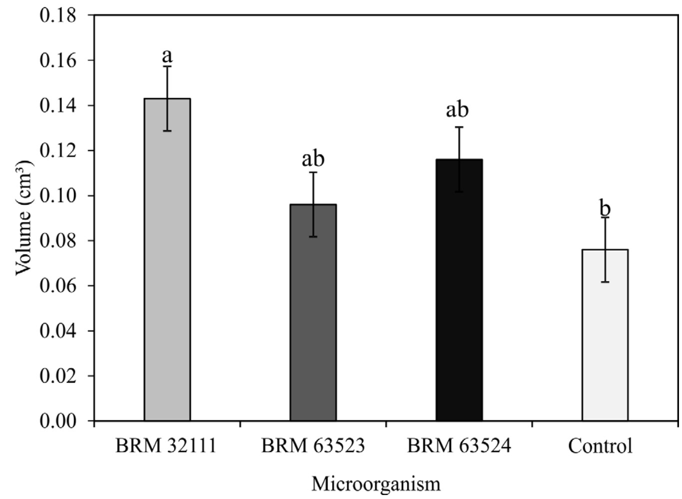

Figure 4. Root volume (VolR) of rice seedlings cultivar BRS 501 CL, as a function of treatment with rhizobacteria: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control (without microorganisms). Evaluation carried out 15 days after sowing in the test tubes.

In relation to principal component analysis, it can be seen that the variability of treatments with isolated microorganisms in relation to total root length (LengR, cm), root diameter (DiamR, mm), total root surface area (AreaS, cm^2), and root volume (VolR, cm^3) of upland rice seedlings treated with multifunctional microorganisms were best described by two principal components (PCs), accounting for 98.67% of the variation in the data, i.e. PC1 (93.5%) and PC2 (5.17%) (Figure 5). The factor map (biplot) shows groups of variables (arrows) denoting positive and negative correlations with each principal component (PC), with the length of the arrow indicating the magnitude of each response for each PC (Figure 5B). For example, PC1 was negatively correlated with all the variables analyzed. On the other hand, PC2 was positively correlated with root diameter, and negatively with total root length, total surface area and root volume.

Based on the representational quality of the treatments with isolated microorganisms, the BRM 32111 treatment showed the highest positive correlation for total root length, total root surface area and root volume (Figure 5). The average root diameter variable was positively correlated with treatment with BRM 63524. The BRM 63524 treatments and the control treatment were not positively correlated with any of the variables analyzed.

The significant increase in the parameters evaluated in the treatments with isolates BRM 32111 and BRM 63524 can be attributed to the high production of phytohormones by the genera to which these microorganisms belong. The treatments that include these rhizobacteria show superior root development when compared to the control treatment, in which these microorganisms are not applied (Figure 6). In this way, inoculation with PGPRs isolates can have positive

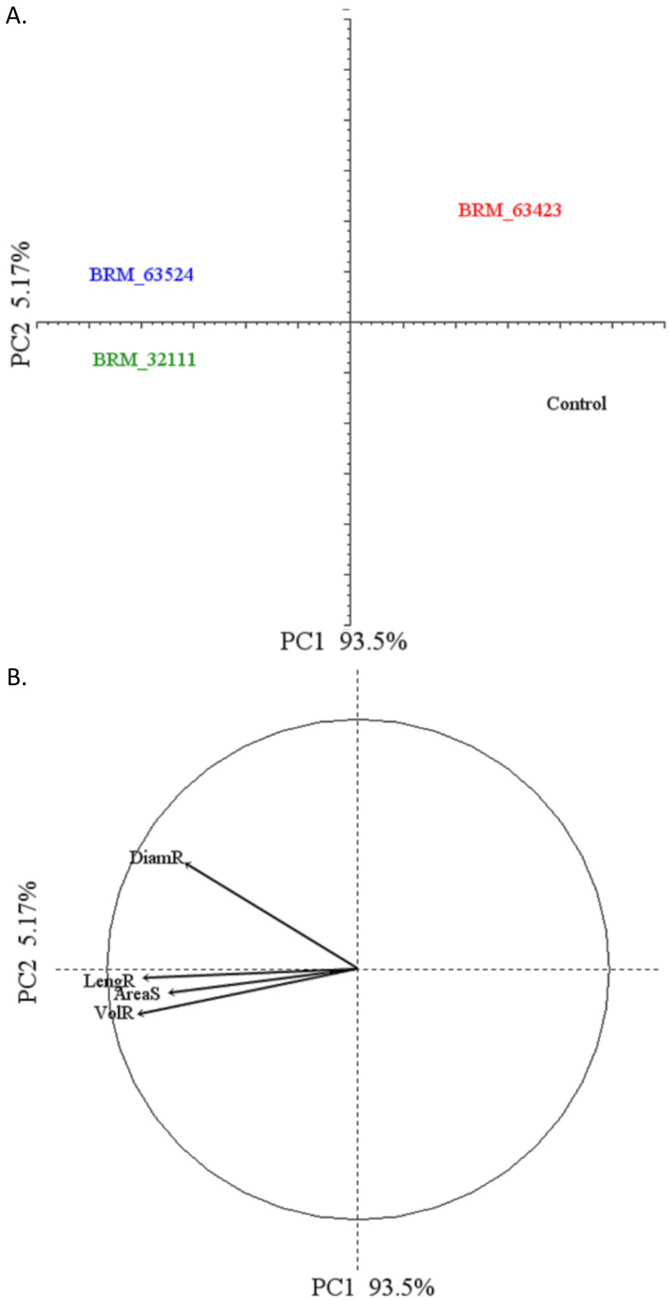

Figure 5. Principal component analysis (PCA) PCA1 × PCA2, explaining the correlations between the variables evaluated and the four treatments with isolated multifunctional microorganisms and the control (without microorganisms). Two principal components (PCs) were responsible for 98.9% of the variation in the data. (A) Biplot graph for treatments: BRM 32111 (*Burkholderia* sp.), BRM 63523 (*Serratia* sp.), BRM 63524 (*Bacillus* sp.), and control treatment (no microorganism). (B) Graph with the correlation circle of the variables: total root length (LengR, cm), average root diameter (DiamR, mm), total root surface area (AreaS, cm^2), and root volume (VolR, cm^3).

effects on upland rice cultivation. This more robust initial development can positively influence various physiological aspects, including the absorption of nutrients, the vigor of corn plants and, potentially, increase crop productivity (Nascente et al., 2017).

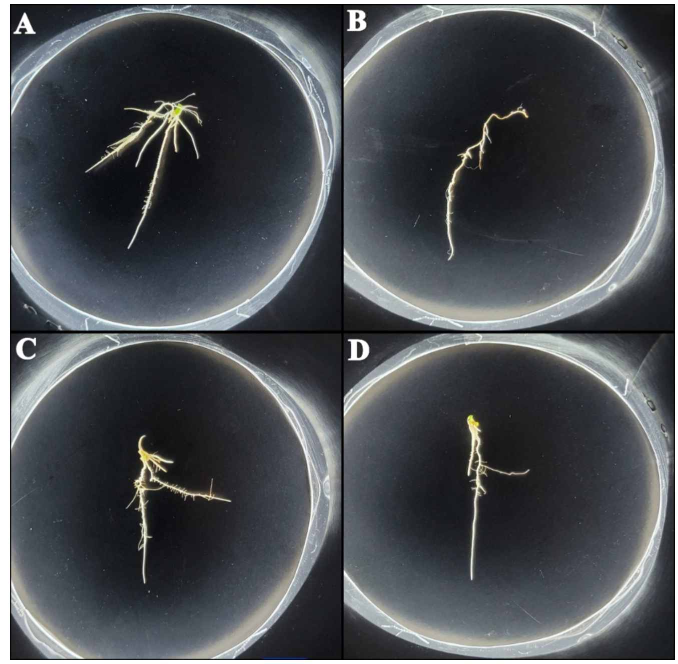

Figure 6. Root system of upland rice seedlings submitted to microbiolization with multifunctional rhizobacteria: (A) BRM 32111 (*Burkholderia* sp.), (B) BRM 63523 (*Serratia* sp.), (C) BRM 63524 (*Bacillus* sp.), and (D) control treatment (no microorganism). The rice seedlings were photographed 15 days after sowing with a digital camera. These images were then processed using WinRHIZO 2008 software in order to gain a deeper understanding of the interactions between microorganisms and plants.

The results obtained in the study indicate that the use of multifunctional microorganisms has promising potential to promote significant benefits in the initial growth of the roots and aerial part of upland rice seedlings. The lack of application of multifunctional rhizobacteria (control treatment) resulted in the worst performance in relation to most of the variables evaluated in the control treatment. However, it is essential to carry out additional studies in laboratory environments, vegetation houses and in the field to examine the implications of these changes on physiological processes and plant development, as well as on crop productivity.

Conclusions

The upland rice seedlings that received treatment with BRM 32111 (*Burkholderia* sp.) and BRM 63524 (*Bacillus* sp.) showed significant increases in the development of the root system compared to the seedlings that did not receive treatment. The control treatment showed the worst results for all the variables analyzed. The use of RPCVs as bioagents is a promising alternative for better root development of rice seedlings.

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Compliance with Ethical Standards

Authors contributions: Conceptualization: DRCC; ASN; NOCM; IVLF; Data curation: DRCC; NOCM; Formal analysis: DRCC; NOCM; IVLF; Funding acquisition: DRCC; ASN; Investigation: DRCC; NOCM; IVLF; RBO; Methodology: DRCC; NOCM; IVLF; RBO; Project administration: DRCC; ASN; Resources: DRCC; ASN; Software: DRCC; NOCM; RBO; Supervision: DRCC; ASN; NOCM; IVLF; Validation: DRCC; ASN; NOCM; IVLF; Visualization: DRCC; ASN; NOCM; IVLF; RBO; Writing – original draft: DRCC; ASN; NOCM; IVLF; RBO; Writing – review & editing: DRCC; ASN; NOCM; IVLF.

Conflict of interest: The authors declare that they have no conflict of interest.

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