







## Effects of isolated and combined growth-promoting microorganisms altering root and shoot development of corn seedlings

Microrganismos promotores de crescimento isolados e combinados alterando o desenvolvimento de raiz e parte aérea de plântulas de milho

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Recebido 29/07/2022

Aceito 27/02/2024

Publicado: 16/04/2024

### Resumo:

Microrganismos multifuncionais podem contribuir significativamente para melhorar o desempenho inicial das plantas, sendo uma biotecnologia inovadora ao buscar o desenvolvimento sustentável. O objetivo deste trabalho foi determinar os efeitos da aplicação de diferentes microrganismos promotores de crescimento, individualmente e em combinação, no desenvolvimento radicular e aéreo de mudas de milho. O experimento foi conduzido em condições controladas em delineamento inteiramente casualizado. Vinte e seis tratamentos consistiram na microbiolização isolada ou combinada de sementes de milho, com as rizobactérias BRM 32109, 32110 e 63573 (*Bacillus* sp.), BRM 32111 e 32112 (*Pseudomonas* sp.), BRM 32113 (*Serratia marcescens*), BRM 32114 (*Serratia* sp.), Ab-V5 (*Azospirillum brasilense*) e BRM 63574 (*Azospirillum* sp.), o fungo BRM 53736 (*Trichoderma koningiopsis*) e um tratamento controle. Os dados obtidos foram analisados por meio de análise de componentes principais ou análise multivariada. Mudas tratadas com BRM 32112, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736, Ab-V5 e BRM 32114 apresentaram aumentos de até 40% no desenvolvimento da raiz e da parte aérea em comparação com mudas não tratadas. O uso de microrganismos multifuncionais é uma alternativa promissora e eficiente para melhor desenvolvimento radicular e aéreo de mudas de milho, sendo a microbiolização uma técnica de inoculação.

**Palavras-chave:** *Zea mays* L, rizobactérias, *Trichoderma*, sustentabilidade, biotecnologia.

### Abstract:

Multifunctional microorganisms can significantly contribute to improve the initial performance of plants, being an innovative biotechnology when seeking sustainable development. The aim of this work was to determine the effects of the application of different growth promoting microorganisms individually and in combination on the root and aerial development of corn seedlings. The experiment was carried out under controlled conditions in a completely randomized design. Twenty-six

treatments consisted of isolated or combined microbiolization of corn seeds, with the rhizobacteria BRM 32109, 32110 and 63573 (*Bacillus* sp.), BRM 32111 and 32112 (*Pseudomonas* sp.), BRM 32113 (*Serratia marcescens*), BRM 32114 (*Serratia* sp.), Ab-V5 (*Azospirillum brasilense*) and BRM 63574 (*Azospirillum* sp.), the fungus BRM 53736 (*Trichoderma koningiopsis*) and a control treatment. The data obtained were analyzed using principal component analysis or multivariate analysis. Seedlings treated with BRM 32112, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736, Ab-V5 and BRM 32114 showed increases of up to 40% in root and shoot development compared to untreated seedlings. The use of multifunctional microorganisms is a promising alternative for better root and aerial development of corn seedlings, and microbiolization is an efficient inoculation technique.

**Keywords:** *Zea mays* L, rhizobacteria, *Trichoderma*, sustainability, biotechnology.

## 1. Introduction

Corn is the most widely planted cereal on the planet grown in all parts of the world, with world production in the 2019/20 crop year of 1.114 billion tons, on an area of 193.733 million planted hectares (Fao, 2021). The corn crop in Brazil in the sum of the productions, related to the first and second harvests 2020/21, reached 117,181.5 thousand tons, representing an increase of 34.6% in relation to the previous harvest, in an area of 20.93 million hectares (Conab, 2021).

The intensification and dependence on the use of synthetic inputs for Brazilian agricultural production results in numerous problems, including environmental problems such as soil, water and air contamination. Thus, it is necessary to search for sustainable technologies that provide a reduction in the use of synthetic inputs, production costs and negative impacts on the environment, thus ensuring the safety and quality of food.

Among the organisms existing in the rhizosphere, the rhizobacteria plant growth promoters (PGPRs) stand out. They are defined as having a high affinity for occupying the rhizospheric environment and are capable of performing activities related to the promotion of plant development (Cardoso & Andreote, 2016). Several PGPRs have been frequently isolated from the rhizosphere of various crop plants and are being studied, such as: *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Azotobacter*, *Staphylococcus* e *Azospirillum* (Mahmood et al., 2016). Some authors state that through various mechanisms such as biological nitrogen fixation (BNF), phosphate solubilization, production of phytohormones, production of siderophores, production of biofilm and biological control PGPRs can generate good yields in plants even with reduced use of synthetic inputs.

Fungi of the genus *Trichoderma* spp. are considered natural from the soil, being able to live saprophytically or parasitizing other fungi and microorganisms, being very well known for their action in the biological control of pests and diseases (Bortolin et al., 2019). In addition, fungi of the genus *Trichoderma* are able to act as biostimulants of root growth, promoting root development through phytohormones and thus improve nutrient assimilation (Machado et al., 2012). Salas-Marina et al. (2011) describe that these fungi are used as inoculants in several agricultural crops, being considered free-living microorganisms and are among the most studied and known biocontrol agents in the world.

A promising and recently explored alternative in Brazil to increase the efficiency of the use of growth promoting microorganisms is the mixture of two or more species, a technique defined as co-inoculation. Co-inoculation with PGPRs, such as *Bradyrhizobium* sp, *Rhizobium* sp, *Azospirillum* sp, *Bacillus* spp., *Serratia* spp. e *Pseudomonas* spp. can favor the development of crops, especially the greater development of the root system, with a greater absorption of water and nutrients and consequently the increase in grain yield (Galindo et al., 2016). Chagas et al. (2017), found in their work that plants co-inoculated with *Bacillus* spp. and *Trichoderma* spp. showed superior results compared to plants without inoculation regarding the accumulation of biomass, demonstrating its

potential as a growth promoter in soybean, cowpea bean, corn and rice crops. Studies at Embrapa Rice and Bean have confirmed the potential use of these bacteria, as it provided significant increments in the productivity of upland rice, both under greenhouse (Nascente *et al.*, 2017) and field conditions (Nascente *et al.*, 2019).

Despite the benefits of the use of beneficial microorganisms in agriculture, there are still few works in corn culture. Thus, the aim of this work was to determine the effects of the application of different growth promoting microorganisms individually and in combination on root and shoot development of corn seedlings.

## 2. Material and methods

The experiment was conducted in a greenhouse at the headquarters of Embrapa Rice and Bean, in Santo Antônio de Goiás, GO. A medium-textured soil from the superficial layer (0.00-0.20 m) was used in the experiment. The chemical characteristics of the soil were determined according to the methods described by Claessen (1997): pH in H<sub>2</sub>O = 5.5; M.O. = 0.7 g dm<sup>-3</sup>; P-Mehlich = 6 mg dm<sup>-3</sup>; K = 0.7 mmolc dm<sup>-3</sup>; Ca<sup>2+</sup> = 9 mmolc dm<sup>-3</sup>; Mg<sup>2+</sup> = 4 mmolc dm<sup>-3</sup>; Al<sup>3+</sup> = 2 mmolc dm<sup>-3</sup>; H + Al = 40 mmolc dm<sup>-3</sup>; SB = 14 mmolc dm<sup>-3</sup>; CTC Potential (T) = 54 mmolc dm<sup>-3</sup>; Effective TCC (t) = 16 mmolc dm<sup>-3</sup>.

The experimental design used was entirely randomized, with 26 treatments and six repetitions. The treatments consisted in the microbiolization of corn seeds, cultivar AG 8088, with different microorganisms identified, stored and preserved in the collection of Multifunctional Microorganisms of Embrapa Rice and Beans, and their combinations in pairs. Of the microorganisms used nine are rhizobacterial isolates: BRM 32109, BRM 32110 and BRM 63573 (*Bacillus* sp.), BRM 32111 and BRM 32112 (*Pseudomonas* sp.), BRM 32113 (*Serratia marcescens*), BRM 32114 (*Serratia* sp.), Ab-V5 (*Azospirillum brasilense*) and BRM 63574 (*Azospirillum* sp.), fungus isolate BRM 53736 (*Trichoderma koningiopsis*) and also used a control treatment, without the application of microorganisms. Table 1 shows the treatments with isolated and pairwise combined microorganisms used in the experiment:

**Table 1.** Distribution of treatments following plant growth promoting microorganisms.

**Tabela 1.** Distribuição dos tratamentos seguindo os microrganismos promotores de crescimento vegetal.

Treatments	Inoculated Microorganisms	Treatments	Inoculated Microorganisms
1	BRM 32109	14	BRM 63573 + BRM 32114
2	BRM 32110	15	BRM 63574 + BRM 32114
3	BRM 32111	16	BRM 63574 + BRM 63573
4	BRM 32112	17	BRM 32110 + BRM Ab-V5
5	BRM 32113	18	BRM 32114 + Ab-V5
6	BRM 32114	19	BRM 63573 + Ab-V5
7	BRM 63573	20	BRM 63574 + Ab-V5
8	BRM 63574	21	Ab-V5 + T-26
9	BRM 53736	22	BRM 32110 + BRM 53736
10	Ab-V5	23	BRM 32114 + BRM 53736
11	BRM 32114 + BRM 32110	24	BRM 63573 + BRM 53736
12	BRM 63573 + BRM 32110	25	BRM 63574 + BRM 53736
13	BRM 63574 + BRM 32110	26	Control

Before microbiolization the seeds were disinfected by immersion in 70% alcohol for two minutes and then in 0.05% sodium hypochlorite for the same time, followed by drying at room temperature at 29 °C. The corn seeds microbiolized with rhizobacteria were submitted to the methodology proposed by Cruz *et al.* (2022), which adapted the methodology of Filippi *et al.* (2011) for the corn crop, of immersing the seeds in bacterial suspensions.

The solution based on *Trichoderma koningiopsis* was made with the dilution of 10 g of fungus spores, cultivated in laboratories under cooked parboiled rice (Silva *et al.*, 2011), in one liter of distilled water. To produce combinations of microorganisms, equivalent amounts of each microorganism involved were added to the microbiolization solution. Corn seeds were immersed in each microorganism suspension, depending on the treatment, and control seeds were immersed in distilled and autoclaved water for a period of 4 hours under constant stirring at 25 °C.

After the microbiolization period the seeds were dried at room temperature and then two seeds were sown per 500 ml beaker containing medium-textured soil from an arable soil layer (0.00-0.20 m), fertilized with 2 g of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O (4-30-10). There was no temperature or relative humidity control in the experimental environment. Daily irrigation of the experimental units was performed, ensuring soil moisture close to field capacity.

At 14 days after planting, the seedlings were removed from the containers and washed under running water to remove excess soil from the roots. After being removed and the excess soil from the roots washed off, the root system of the seedlings was separated from the aerial part and photographed with the help of a digital camera under a black cloth. The images of the seedling root system were analyzed using WinRhizo Pro 2012<sup>®</sup>, performing the following analyses: total root length (LengR, cm), root diameter (DiamR, mm), total root surface area (AreaS, cm<sup>2</sup>) and Root volume (VolR, cm<sup>3</sup>).

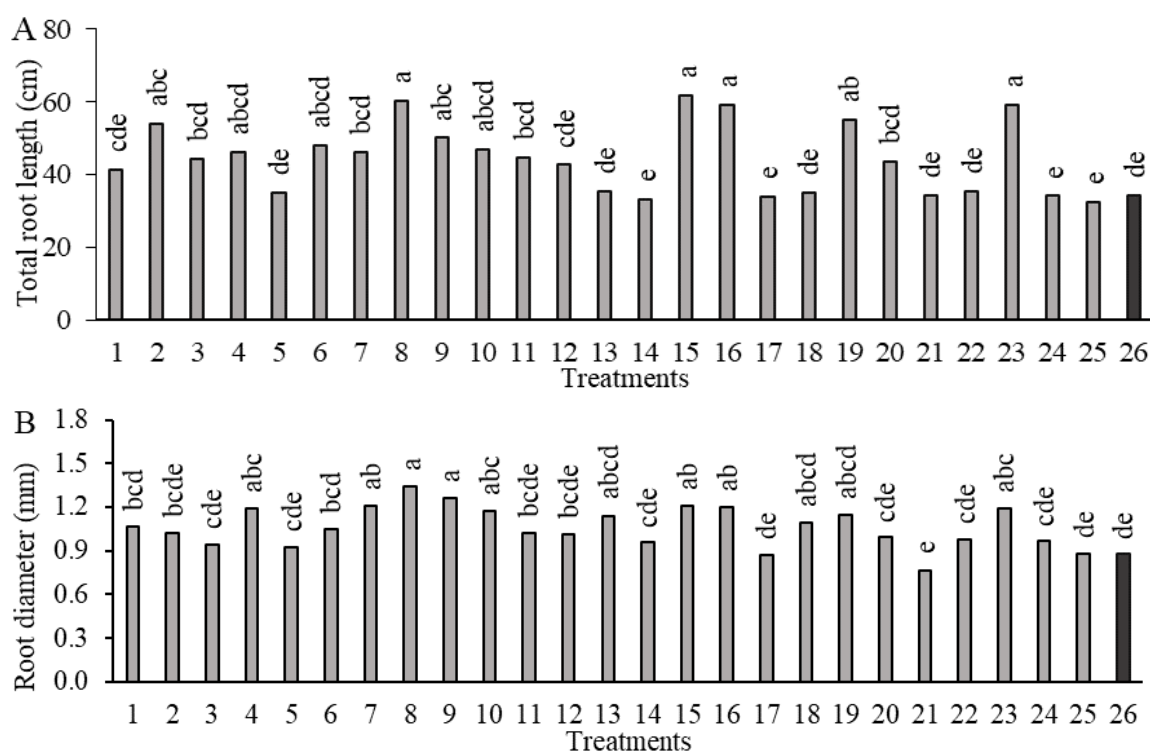
The photographed roots and shoots of the seedlings were placed separately in identified kraft paper bags and dried in a forced ventilation oven at 65 °C for a period of 72 hours. After drying, the material from each envelope was weighed in order to determine root dry mass (RDM), shoot dry mass (SDM), and total (root + shoot) dry mass (TDM), using a precision balance of four decimal places (0.0001). The data obtained were submitted to the analysis of variance by the SAS statistical software and the means were compared by the LSD test ( $p \leq 0.05$ ). Principal components (PCs) were used as response variables when the correlation test result yielded  $r \geq 0.50$ . The first two PCs accounted for 87.4% of the variation in the data. The biplot (two-dimensional graph) correlated the isolated microorganisms and the response variables, and the statistical software R.

### 3. Results and discussion

According to the analysis of variance, there were statistically significant differences among treatments for total root length, root diameter, total root surface area, root volume, root dry mass, shoot dry mass, and total dry mass of corn seedlings treated with multifunctional microorganisms when applied alone or in mixture (Figures 1 to 4). Seedlings treated with the isolate BRM 63574 and the combinations BRM 63574 + BRM 32114, BRM 63574 + BRM 63573 and BRM 32114 + BRM 53736 showed significantly higher values than the control for total root length (Figure 1A). These treatments have in common the fact that they involve genera of microorganisms known for their ability to produce growth phytohormones, especially auxins (IAA), such as the rhizobacteria *Azospirillum* (Sivasakthivelan *et al.*, 2023), *Bacillus* (Xiang *et al.*, 2023), *Serratia* (Jagtap *et al.*, 2023) and the fungus genus *Trichoderma* (Contreras-Cornejo *et al.*, 2023).

**Figure 1.** (A) Total root length (CV: 25.57%) and (B) Root diameter (CV: 27.48%) of corn seedlings, cultivar AG 8088, as a function of treatment with multifunctional microorganisms, used alone or in mixture. Evaluation performed 14 days after corn sowing. \*Values followed by the same letter in the columns do not differ by LSD test ( $p < 0.05$ ). \*\*Treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Control (no microorganisms).

**Figura 1.** (A) Comprimento total de raiz (CV: 25,57%) e (B) Diâmetro de raiz (CV: 27,48%) de plântulas de milho, cultivar AG 8088, em função do tratamento com microrganismos multifuncionais, em uso isolado ou em mistura. Avaliação realizada 14 dias após a semeadura do milho. \*Valores seguidos da mesma letra nas colunas não diferem pelo teste de LSD. \*\*Tratamentos: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Controle (sem microrganismos).



Several microorganisms, such as bacteria and fungi in the soil and or associated with plants, synthesize growth hormones identical to those found in plants, among them IAA, which can contribute to the elongation of roots, which reach a greater length, allowing plants to better exploit the resources available in the soil. Co-inoculation with PGPRs, such as *Bradyrhizobium* sp, *Rhizobium* sp, *Azospirillum* sp, *Bacillus* spp., *Serratia* spp. and *Pseudomonas* spp. can favor the development of crops, especially the greater development of the root system, with a greater absorption of water and nutrients and consequently an increase in grain yield (Galindo *et al.*, 2016). The isolates BRM 32110 and BRM 53736 also showed results, superior to those obtained without the application of microorganisms.

Regarding root diameter the best results were observed in the treatments with BRM 63574, BRM 53736, BRM 63573, BRM 63574 + BRM 32114 and BRM 63574 + BRM 63573 (Figure 1B). As with total root length, the IAA production capacity may have positively influenced the increase in root diameter of seedlings treated with these microorganisms. The phytohormone IAA is known to enhance plant growth by increasing cell elongation and differentiation and stimulating the formation of thicker roots (Melo Júnior, 2020). Larger average root diameters do not always mean a benefit to the seedlings, because an increase in fine roots and a reduction in coarse roots tends to increase nutrient uptake, since root hairs are responsible for greater absorption (Souto et al., 2016). However, the fact that they also present higher results for total length (Figure 2A), denotes that there was greater production of secondary roots and development of roots. Cosme & Wurst (2013) afirmam que a utilização de rizobactérias e fungos promotores de crescimento altera a morfologia das raízes, aumentando o volume e comprimento de raízes secundárias, melhorando a aquisição de águas e nutrientes.

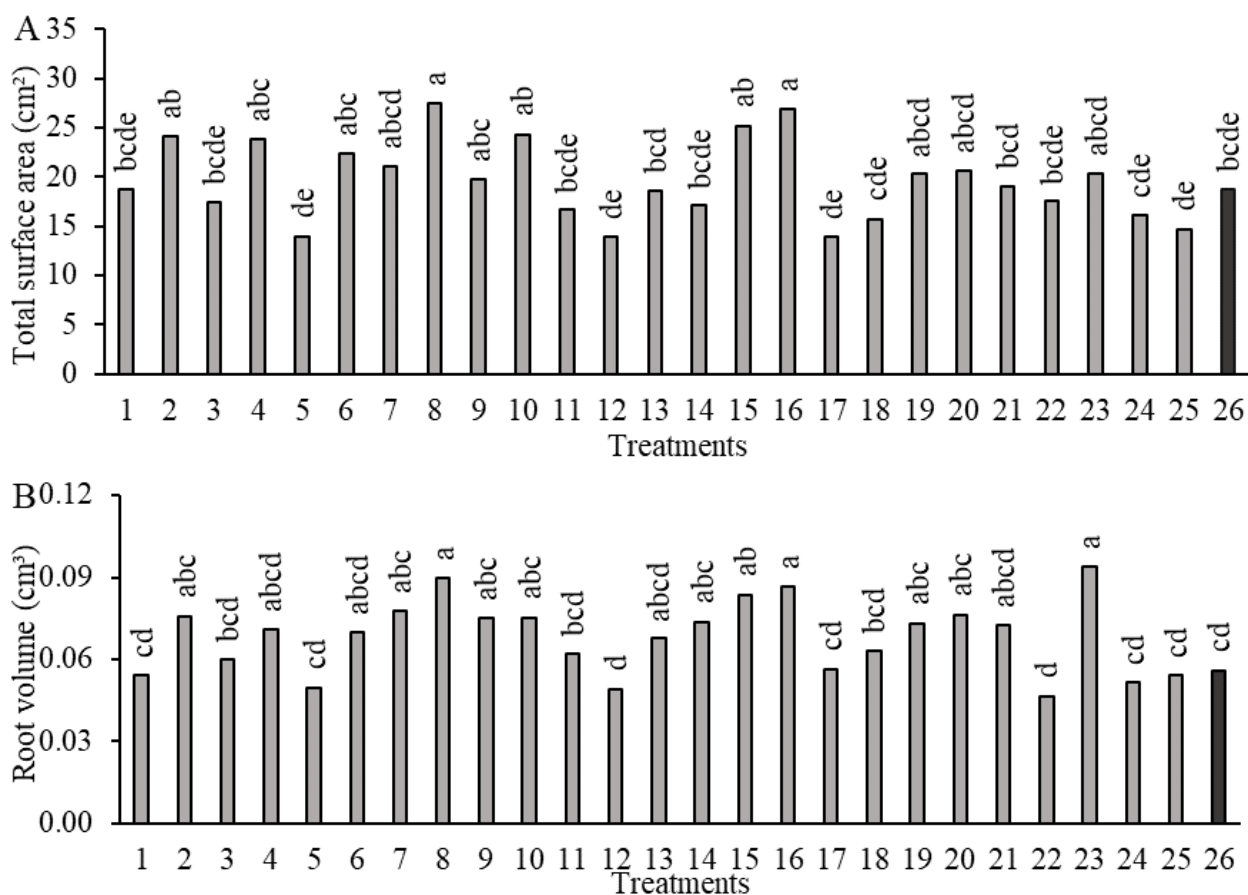
The isolate BRM 63574 and the combination BRM 63574 + BRM 63573, were the only treatments that provided significant increases in total root surface area, differing statistically from the control treatment (Figure 2A). These increases were 47.02% for BRM 63574 and 43.88% for BRM 63574 + BRM 63573, compared to the control treatment. Auxin production leads to greater proliferation of lateral roots and increased root hair formation, resulting in greater total root surface area and increased nutrient and water uptake by the plant (Gomes et al., 2016). The PGPRs can generate an increase in the plant's root system and nutrient and water uptake by producing phytohormones such as IAA, which is necessary for cell division and elongation in plants (Tirry et al., 2021). In addition to auxin production by these microorganisms, one can also highlight the ability to solubilize phosphorus by isolate BRM 63573, which belongs to the genus *Bacillus* sp. and isolate BRM 63574, from the genus *Azospirillum* sp.. Besides favoring the solubilization of natural phosphates, rhizobacteria can increase the efficiency of soluble sources (Guimarães et al., 2021), positively influencing the root growth of the host plant (Mhatre et al., 2019).

The largest root volumes were observed in the treatments BRM 63574, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736 and BRM 63574 + BRM 63573 (Figure 2B). As can be seen the variables length, surface area and total root volume are dependent on each other, so they show similar trends among the microorganisms that stand out. Thus, higher values obtained for total root length and surface area lead consequently to a greater total root volume, characteristics that facilitate the uptake of water and nutrients by plants, mainly through increased root development, resulting in greater access to and use of resources (Ahemad & Kibret, 2014).

The seedlings treated with BRM 32112, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, Ab-V5 and BRM 32114 + BRM 53736 obtained the highest values for shoot dry mass, with statistically superior results to the control treatment (Figure 3A). The increase in shoot dry mass may be related mainly to better plant nutrition, which may be motivated by root growth through the action of phytohormones and also the greater absorption of nutrients. The main effect of phosphate solubilization on plant growth promotion is in increasing plant biomass and P content (Guimarães *et al.*, 2021), may be one of the main reasons for the greater accumulation of dry mass in the shoots, since the highlighted microorganisms possess this ability (Silva *et al.*, 2020). Improved plant growth in terms of seed germination, shoot and root development, increased biomass, and reduced disease incidence is observed as a result of the application of biotechnologies based on plant growth-promoting microorganisms (Cortivo et al., 2020, Pathania et al. 2020).

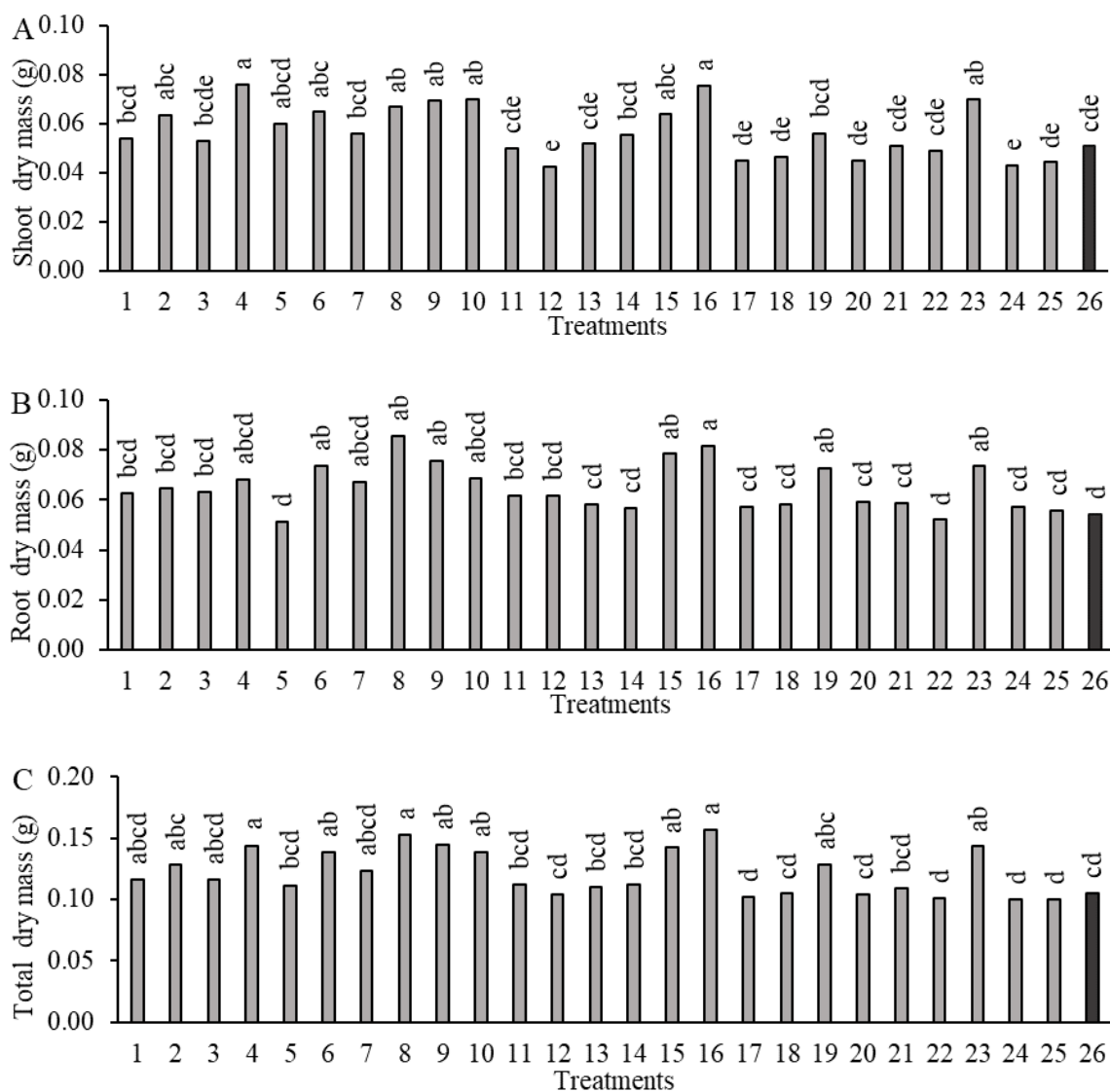
**Figure 2.** (A) Total surface area (CV: 27.28%) and (B) Root volume (CV: 24.48%) of corn seedlings, cultivar AG 8088, as a function of treatment with multifunctional microorganisms, used alone or in mixture. Evaluation performed 14 days after corn sowing. \*Values followed by the same letter in the columns do not differ by LSD test ( $p < 0.05$ ). \*\*Treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Control (no microorganisms).

**Figura 2.** (A) Área de superfície total (CV: 27,28%) e (B) Volume de raízes (CV: 24,48%) de plântulas de milho, cultivar AG 8088, em função do tratamento com microrganismos multifuncionais, em uso isolado ou em mistura. Avaliação realizada 14 dias após a semeadura do milho. \*Valores seguidos da mesma letra nas colunas não diferem pelo teste de LSD. \*\*Tratamentos: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Controle (sem microrganismos).



**Figure 3.** (A) Shoot dry mass (CV: 14.44%), (B) Root dry mass (CV: 25.68%) and (C) Total dry mass (CV: 22.40%) of corn seedlings, cultivar AG 8088, as a function of treatment with multifunctional microorganisms, used alone or in mixture. Evaluation performed 14 days after corn sowing. \*Values followed by the same letter in the columns do not differ by LSD test ( $p < 0.05$ ). \*\*Treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Control (no microorganisms).

**Figura 3.** (A) Massa seca de parte aérea (CV: 14,44%), (B) Massa seca de raiz (CV: 25,68%) e (C) Massa seca total (CV: 22,40%) de plântulas de milho, cultivar AG 8088, em função do tratamento com microrganismos multifuncionais, em uso isolado ou em mistura. Avaliação realizada 14 dias após a semeadura do milho. \*Valores seguidos da mesma letra nas colunas não diferem pelo teste de LSD. \*\*Tratamentos: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Controle (sem microrganismos).





The treatments with, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736, BRM 63573 + Ab-V5 and BRM 32114 showed the highest values of root dry mass, differing statistically from the non-application of microorganisms (Figure 3B). The greater accumulation of root mass may be linked to the ability of these microorganisms to act by producing growth phytohormones, such as auxins and gibberellins, pectinase, and also show that they are able to increase the availability of phosphorus in the soil (Zucareli *et al.*, 2018). The IAA-producing bacteria can enhance plant growth, especially root initiation and elongation, resulting in greater root mass (Shao *et al.*, 2015). With a greater elongation of the roots by the action of phytohormones and increase of the soil exploration zone, naturally occurs a greater absorption and accumulation of nutrients in the root tissues, increasing the dry mass.

In the evaluation of the total dry mass of corn seedlings the treatments that stood out the most were with BRM 32112, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736, Ab-V5 and BRM 32114 (Figure 3C). The shoot dry mass showed a similar behavior to the root dry mass in its values, denoting that root development directly influences the accumulation of nutrients in the aboveground tissues. Consequently, as the total mass is the sum of shoot and root masses, it followed the same trend in relation to the best treatments in this parameter. The significant increases in biomass production and productivity of crops through the use of beneficial microorganisms have among the main explanations, the increase in nutrient uptake and production of phytohormones (Chagas *et al.*, 2016).

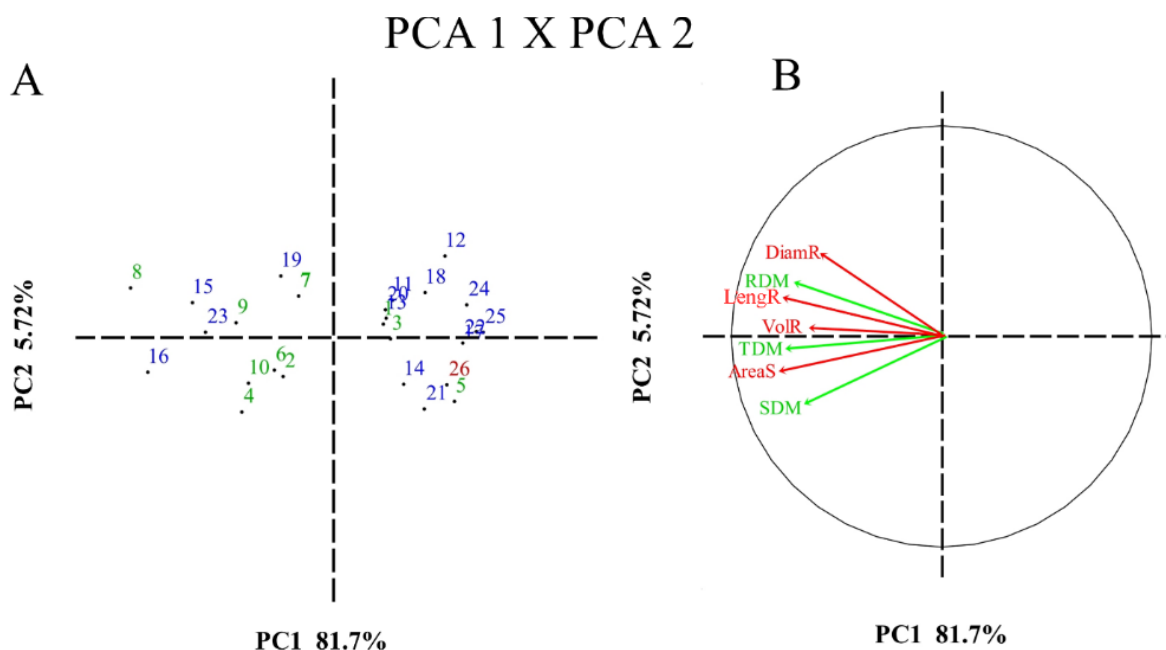
With principal component analysis, it can be seen that the variability of treatments with isolated and combined microorganisms in relation to total root length, root diameter, total root surface area, root volume and dry mass of corn seedlings treated with multifunctional microorganisms was best described by two principal components (PCs), representing 86.42% of the data variation, therefore PC1 (81.70%), PC2 (5.72%) (Figure 4). 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 4B). For example, PC1 was negatively correlated with all the variables analyzed. On the other hand, PC2 was positively correlated with total root length, root dry mass, root diameter and root volume, and negatively correlated with total surface area, shoot dry mass and total root dry mass.

Based on the representational quality of the treatments with microorganisms isolates and in combination for the variables analyzed, treatments 7 (BRM 63573), 8 (BRM 63574), 9 (BRM 53736), 15 (BRM 63574 + BRM 32114), 19 (BRM 63573 + Ab-V5) and 23 (BRM 32114 + BRM 53736) obtained the highest positive correlation for total root length, root dry mass, root diameter and root volume (Figure 4). The variables total surface area, shoot dry mass and total root dry mass correlated positively with treatments 2 (BRM 32110), 4 (BRM 32112), 6 (BRM 32114), 10 (Ab-V5) and 16 (BRM 63574 + BRM 63573). The other treatments did not correlate positively with any of the variables analyzed.

The results obtained in this work demonstrate a promising potential of using multifunctional microorganisms to promote benefits in the initial growth of roots and aboveground parts of corn seedlings. A better initial development of the seedlings can generate an increase in the physiological points of view, in the nutrient absorption, vigor of the corn plants and even in the productivity of the crop (Nascente *et al.*, 2017). In this context, the microorganisms that most positively affected the initial growth and development of corn seedlings were BRM 32112, BRM 63574 + BRM 63573, BRM 63574, BRM 53736, BRM 63574 + BRM 32114, BRM 32114 + BRM 53736, Ab-V5 and BRM 32114. Works in this direction have great appeal worldwide, mainly because it is a technology of easy application and use, non-polluting and inserted in a desirable context to the sustainable intensification of modern agriculture (Silva *et al.*, 2020).

**Figure 4.** Principal component analysis (PCA) PCA1 X PCA2, explaining the correlations between the evaluated variables and 26 treatments with multifunctional microorganisms and the control (without the microorganism). **A.** Biplot plot for treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Control (no microorganisms). The microbial isolates are highlighted in green and the co-inoculations in blue. **B.** Graph with the correlation circle of the variables.

**Figura 4.** Análise de componentes principais (PCA) PCA1 X PCA2, explicando as correlações entre as variáveis avaliadas e 26 tratamentos com microrganismos multifuncionais e o controle (sem o microrganismo). **A.** Gráfico biplot para tratamentos: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 32113; (6) BRM 32114; (7) BRM 63573; (8) BRM 63574; (9) BRM 53736; (10) Ab-V5; (11) BRM 32114 + BRM 32110; (12) BRM 63573 + BRM 32110; (13) BRM 63574 + BRM 32110; (14) BRM 63573 + BRM 32114; (15) BRM 63574 + BRM 32114; (16) BRM 63574 + BRM 63573; (17) BRM 32110 + Ab-V5; (18) BRM 32114 + Ab-V5; (19) BRM 63573 + Ab-V5; (20) BRM 63574 + Ab-V5; (21) Ab-V5 + BRM 53736; (22) BRM 32110 + BRM 53736; (23) BRM 32114 + BRM 53736; (24) BRM 63573 + BRM 53736; (25) BRM 63574 + BRM 53736; (26) Controle (sem microrganismos) e **B.** Gráfico com o círculo de correlação das variáveis.



#### 4. Conclusions

Corn seedlings treated with the isolates BRM 32112, BRM 63574, BRM 32114, BRM 53736 and Ab-V5, along with the combinations BRM 63574 + BRM 63573, BRM 63574 + BRM 32114 and BRM 32114 + BRM 53736, had greater development in the root and shoot system compared to untreated seedlings. Therefore, the use of multifunctional microorganisms in isolation and in mixtures is a promising alternative for better root and aerial development of corn seedlings.

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**Acknowledgment**

This work was supported by Universidade Federal de Goiás (UFG), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa e ao Desenvolvimento (FAPED) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

**Financing Sources**

There was no source of funding.

**Conflict of Interest**

The authors declare no conflict of interest.

**Associate Editor**

Saly Takeshita Yamaguti

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ORIGINAL ARTICLE

