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Root and shoot development of corn seedlings as affected by rhizobacteria

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Abstract

Multifunctional microorganisms are beneficial microorganisms able of promoting plant growth through direct and indirect mechanisms. Because of this, has enormous potential for use when aiming a sustainable agriculture. The objective of this work was to determine the effect of seed inoculation with multifunctional microorganisms on the initial development of corn seedlings. The experiment was carried out under controlled conditions in a completely randomized design, with seven treatments and six replications. Treatments consisted of microbiolization of corn seeds with the rhizobacteria: 1. *Burkholderia cepacea* (BRM 32111), 2. *Serratia marcenses* (BRM 32113), 3. *Serratia* sp. (BRM 32114), 4. *Bacillus* sp. (BRM 63573), 5. *Azospirillum brasilense* (Ab-V5), 6. *Azospirillum* sp. (BRM 63574) and 7. control treatment (no microorganisms). For each experimental unit, 500 mL plastic pots filled with soil and two corn seeds were used. After 14 days the seedlings were removed from the pots, set aside from the ground and photographed with a digital camera. The images presented were analyzed by WinRHIZO 2012 software to determine: total root length, root diameter, total root surface area and root volume. After this, roots and shoots of the seedlings were dried and weighed. Corn seedlings treated with the multifunctional microorganisms *Bacillus* sp. (BRM 63573), *Serratia* sp. (BRM32114) and *Azospirillum* sp. (BRM 63574) presented increased in the root and shoots biomass compared to untreated seeds. The microorganism *Azospirillum* sp. (BRM 63574) was the one that provided the highest values in the parameters of total root length, root diameter, root volume, root dry mass, shoot dry mass and total dry mass compared to the control treatment. The use of multifunctional microorganisms is a promising alternative to provide greater development of corn seedlings.

Keywords: *Zea mays* L.; multifunctional microorganisms; sustainability; plant growth-promoting.

Desenvolvimento radicular e aéreo de plântulas de milho afetado por rizobactérias

Resumo

Microrganismos multifuncionais são microrganismos benéficos, capazes de promover o crescimento vegetal por meio de ações diretas e indiretas, tendo, portanto, enorme potencial de uso quando se visa a agricultura sustentável. O objetivo deste trabalho foi determinar o efeito da inoculação de sementes com microrganismos multifuncionais no desenvolvimento inicial de plântulas de milho. O experimento foi conduzido em condições controladas em delineamento inteiramente casualizado, com sete tratamentos e seis repetições. Os tratamentos consistiram na microbiolização das sementes de milho, cultivar AG 8088, com os isolados de rizobactérias: 1. *Burkholderia cepacea* (BRM 32111), 2. *Serratia marcescens* (BRM 32113), 3. *Serratia* sp. (BRM 32114), 4. *Bacillus* sp. (BRM 63573), 5. *Azospirillum brasilense* (Ab-V5), 6. *Azospirillum* sp. (BRM 63574) e 7. tratamento controle (sem microrganismos). Para cada unidade experimental foram utilizados copos plásticos de 500 mL, preenchidos com solo e com duas sementes de milho. Após 14 dias as plântulas foram retiradas dos copos, separadas do solo e fotografadas com câmera digital. As imagens obtidas foram analisadas pelo software WinRHIZO 2012, para determinação de: comprimento total da raiz, diâmetro da raiz, área de superfície total de raízes e volume de raízes. Após serem fotografadas as raízes e parte aérea das plântulas foram levadas a estufa de secagem até atingirem massa constante e pesadas. As plântulas de milho tratadas com os microrganismos multifuncionais *Bacillus* sp. (BRM 63573), *Serratia* sp. (BRM32114) e *Azospirillum* sp. (BRM 63574) apresentaram incremento do sistema radicular e aéreo em comparação com plântulas não tradadas. O microrganismo *Azospirillum* sp.

(BRM 63574) foi o que proporcionou maiores valores nos parâmetros de comprimento total da raiz, diâmetro da raiz, volume da raiz, massa seca da raiz, massa seca de parte aérea e massa seca total em comparação ao tratamento controle. A utilização de microrganismos multifuncionais é alternativa promissora para proporcionar maior desenvolvimento de plântulas de milho.

Palavras-chave: *Zea mays* L.; microrganismos multifuncionais; sustentabilidade; promotoras de crescimento de plantas.

Introduction

Corn is the most produced cereal in the world, due to its high production and, mainly, its wide use (OLIVEIRA, 2019). The crop is one of the most important agricultural commodities worldwide, and it is the main energy source for animal feed, in the cuisine of several countries, and an important source in the production of renewable fuel (CONAB, 2019). The world's largest producers are the United States, China and Brazil, which produce approximately 392, 257 and 82 million tons, respectively (FAO, 2021).

Corn is an important product in the Brazilian agriculture, and it is produced practically throughout the country, as an important source of income and with good participation in the national economy (MIRANDA, 2018). The current production model most used for this crop is based on excessive applications of pesticides and synthetic fertilizers, generating severe problems for the environment and also financial impacts. These damages caused by the intensive use of synthetic fertilizers, herbicides, fungicides and insecticides show the need to seek alternatives that provide the sustainable development of agriculture (LOPES; ALBUQUERQUE, 2018). According to Silva *et al.* (2020), among the existing alternatives to increase the efficiency of the use of chemical fertilizers and reduce the amount applied in agricultural production environments, the use of microorganisms that promote plant growth can be highlighted.

Among the microorganisms selected in the rhizosphere, plant growth-promoting rhizobacteria (PGPR) stand out, which have a high affinity for ocpotying the rhizospheric environment and are capable of performing activities related to promoting plant development (CARDOSO; ANDREOTE, 2016). This plant growth promotion is linked to the ability of this group of microorganisms to act positively on physiological growth characteristics, using different mechanisms of action, such as nutrient solubilization, inhibition of pest and plant pathogen development, production of bactericides, antifungals, growth hormones, siderophores and biological nitrogen fixation (LAKSHMANAN *et al.*, 2015). Several plant growth-promoting rhizobacteria have been frequently isolated from the rhizosphere of several cultivated plants and have been studied, such as: *Agrobacterium, Arthrobacter, Bacillus, Burkholderia, Pseudomonas, Serratia, Azotobacter, Staphylococcus e Azospirillum* (MAHMOOD *et al.*, 2016).

In this context, the use of multifunctional microorganisms represents a biotechnology option whose objective is to promote plant growth by promoting greater development of the root system, increasing nutrient absorption, gas exchange efficiency and, consequently, phytomass production and grain yield (NASCENTE *et al.*, 2017). The greater root development promoted by multifunctional microorganisms can also promote, in addition to greater use of nutrients, easier access to water (GLICK, 2012). Several studies report greater plant development with the use of multifunctional microorganisms and cite the production of hormones as one of the possible causes for this greater development in rice cultivation (SPERANDIO *et al.*, 2017; SOUSA *et al.*, 2019; FERNANDES *et al.*, 2021), wheat (SPAEPEN *et al.*, 2008) and corn (RAZA; FAISAL, 2013; NAVEED *et al.*, 2014), demonstrating the potential use of this biotechnology in the Poaceae family.

Studies done by researchers of Embrapa Rice and Bean allowed the identification of beneficial rhizobacteria collected in upland rice fields (FILIPPI *et al.*, 2011). After selection and characterization, greenhouse studies confirmed the potential use of these multifunctional microorganisms, which promoted significant increases in gas exchange and in the production of aboveground biomass of upland and irrigated rice plants (NASCENTE *et al.*, 2017). Therefore, it became interesting to carry out studies to evaluate the use of these isolates in different cultures of great importance, as has already been done in upland rice, irrigated rice, common beans and soybeans (this included the work of the staff). Thus, it is also possible that these microorganisms positively affect the development of corn plants. The objective of this work was to determine the effect of seed inoculation with rhizobacteria on root and shoots development of corn seedlings.

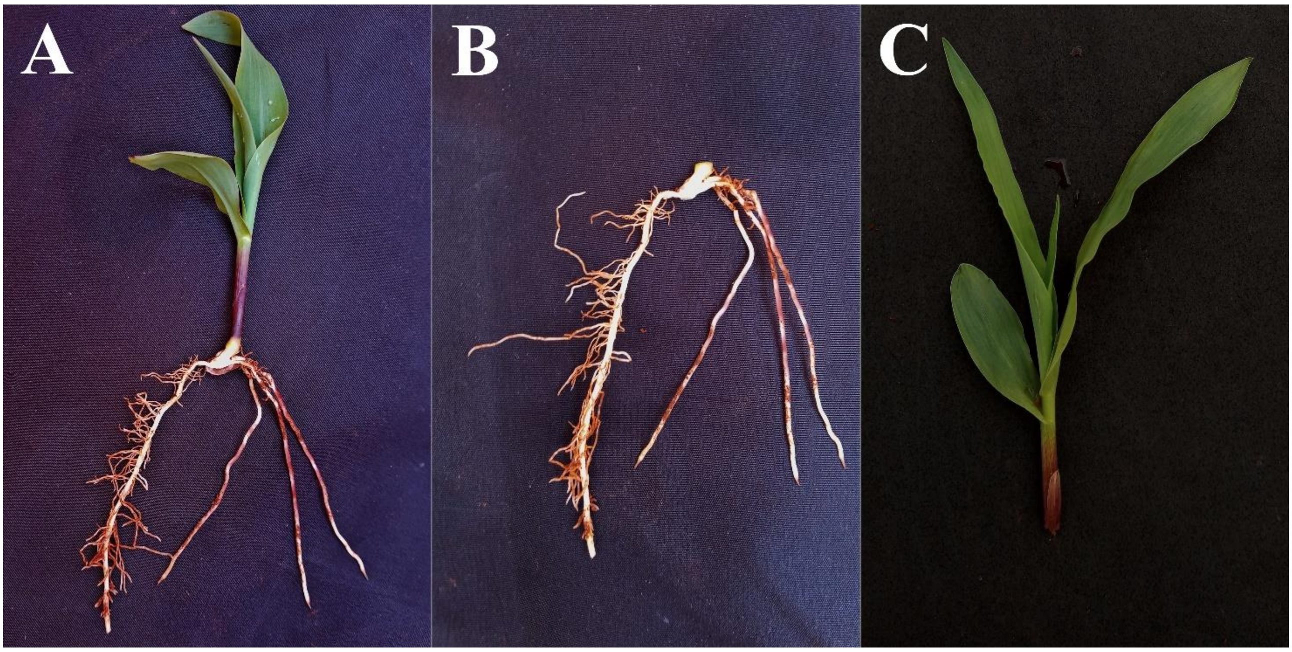
Material and methods

The experiment was carried out in a greenhouse, under controlled conditions, at the headquarters of Embrapa Rice and Bean, in Santo Antônio de Goiás, GO. The experimental design used was completely randomized, with seven treatments and six replications, totaling 42 experimental plots. The treatments consisted of microbiolization of corn seeds, cultivar AG 8088, with six rhizobacteria isolates: BRM 63573 (*Bacillus* sp.), BRM 32111 (*Burkholderia cepacea*), BRM 32113 (*Serratia marcenses*), BRM 32114 (*Serratia* sp.), Ab-V5 (*Azospirillum brasilense*) e BRM 63574 (*Azospirillum* sp.). A control treatment was also included, without the application of microorganisms. These microorganisms are identified, stored and preserved in the collection of Multifunctional Microorganisms at Embrapa Rice and Bean.

For the microbiolization of corn seeds, the methodology proposed by Filippi *et al.* (2011), immersion of seeds in bacterial suspensions. Rhizobacteria were cultivated in solid medium (nutrient agar) and suspensions were prepared in 523 liquid medium (nutrient broth) (KADO; HESKETT, 1970) in a shaking incubator for 24 hours at 28 °C. Afterwards, the concentration was fixed in a spectrophotometer to A540 = 0.5 (10^8 CFU (colony forming unit)). Before microbiolization, the seeds were disinfested 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. Corn seeds were immersed in each suspension of microorganisms, depending on the treatment, and control seeds were immersed in distilled water and autoclaved for a period of 4 hours under constant agitation at 25 °C. The period of microbiolization was determined by means of a test performed previously with Ab-V5 (*Azospirillum brasilense*), which, when testing the microbiolization of seeds in different periods, led to the conclusion that the best seedling development was observed with the period of microbiolization 4 hours.

For each experimental unit, 500 mL plastic pots were used, filled with medium textured soil (Oxisol) from the surface layer (0.00-0.20 m), where two seeds were sown per pot. Before planting, 2 g of N-P_2O_5-K_2O (5-30-15) were applied to each pot. Although the experiment was carried out in a greenhouse, temperature and relative humidity control were not controlled. Daily irrigations were carried out, keeping the soil moisture of the experimental units always at field capacity. After 14 days, the soil of each pot was washed to evaluate the root system of the corn seedlings, where the most vigorous seedling of each pot was evaluated. The removed seedlings were photographed with a digital camera and the necessary image processing was performed (Figure 1). The images obtained were analyzed using the software WinRHIZO 2012, Regent Instruments, Inc. Quebec City, QC, Canada (ARSENAULT *et al.*, 1995). The following analyzes were performed using the software: total root length (LengR , cm), root diameter (DiamR, mm), total root surface area (AreaS, cm^2) and root volume (VolR, cm^3).

Figure 1. Images of the root and shoot systems of corn seedlings microbiolized with rhizobacteria 14 days after sowing: **A.** Complete seedling, **B.** Root and C. Shoot. Source: Personal Archive (2021).



After photographing the seedlings, roots and the shoots were placed separately in kraft-type paper envelopes, properly identified, and were dried in a forced ventilation oven, at 65 ºC, until constant mass. Then, the material in each envelope was considered 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 subjected to analysis of variance by the SAS statistical software and, when significant, the means were compared using the Tukey test ($p \leq 0.05$). Principal components (PCs) were used as response variables when the result of the correlation test produced $r \geq 0.50$. The biplot (two-dimensional graph) correlated the isolated microorganisms and the response variables, and for this purpose, the statistical software R was used.

Results and discussion

Compared to the control treatment (no application of microorganisms), treatments with application of rhizobacteria presented statistically higher values in the evaluated parameters (Table 1). The roots of seedlings treated with *Azospirillum* sp. (BRM 63574) were the ones with the greatest total root length, with a superiority of 43.71% in relation to the control treatment. According to Kaushal (2019), inoculation by *Azospirillum* sp. can generate positive morphological changes to the root system through the production of substances that regulate plant growth, such as phytohormones, increasing, for example, the length of roots and the capacity to explore the soil. According to Cassán and Diaz-Zorita (2016), the production of phytohormones, mainly auxins, by the genus *Azospirillum* sp. is its main characteristic for promoting plant growth, precisely because it promotes an increase in root length and volume. Treatments with *Burkholderia cepacea* (BRM 32111) and *Bacillus* sp. (BRM 63573) also showed good results and were superior in total root length compared to the control treatment (Table 1). PGPRs can promote changes in the root morphology of corn plants, mainly in terms of length increase, which may reflect in greater soil exploration and nutrient uptake (DAR *et al.*, 2018).

Table 1. Total root length (LengR), root diameter (DiamR), Total root surface area (AreaS) and root volume (VolR) of seedlings of corn, cultivar AG 8088, as a function of treatment with rhizobacteria. Evaluation carried out 14 days after corn sowing.

Treatments	LengR	DiamR	AreaS	VolR
	(cm)	(mm)	(cm^2)	(cm^3)
Serratia marcenses (BRM 32113)	34.91 bc	0.63 ab	13.94 c	0.037 c
Burkholderia cepacea (BRM 32111)	39.90 ab	0.64 ab	14.86 bc	0.048 bc
Serratia sp. (BRM32114)	37.97 bc	0.76 a	19.26 ab	0.068 a
Bacillus sp. (BRM 63573)	38.47 ab	0.75 a	23.84 a	0.055 ab
Azospirillum brasilense (Ab-V5)	33.92 c	0.68 ab	15.07 bc	0.045 bc
Azospirillum sp. (BRM 63574)	50.30 a	0.82 a	17.25 ab	0.063 a
Control	35.00b c	0.48 b	12.72 c	0.033c
CV (%)	27.80	23.45	28.60	11.43

* Means followed by the same letter in the column do not differ by Tukey's test (p≤0.05).

Seedlings treated with *Serratia* sp. (BRM32114), *Bacillus* sp. (BRM 63573) and *Azospirillum* sp. (BRM 63574) were the ones that showed the greatest increase in relation to the mean diameter of roots compared to the control treatment (Table 1). The genus *Bacillus* sp. is very important among the different PGPRs genera, and was successfully used for several decades with significant effects in plant growth promotion, directly influencing root morphology, due to abilities such as auxin production, nitrogen fixation, phosphorus solubilization and antifungal activity (MILANI *et al.*, 2019). The genus *Serratia* sp. is able to act in the production of growth hormones (CARVALHO *et al.*, 2009) and phosphate solubilization (HAMEEDA *et al.*, 2008), which are mechanisms that greatly assist in root growth and development.

In relation to the total surface area of roots, a greater increase was observed in the values of corn plants treated with *Bacillus* sp. (BRM 63573), which had 87.42% higher total root surface area compared to the control treatment (Table 1). These results corroborate those found by Sousa *et al.* (2018), who found that the microbiolization of corn seeds with strains of *Bacillus* sp. promoted an increase in the total surface area of roots, allowing for greater nutrient uptake and plant growth. Seedlings treated with *Serratia* sp. (BRM32114) and *Azospirillum* sp. (BRM 63574) also showed good results for the variable total surface area of roots, and was statistically superior to the control treatment. The root surface area is a parameter that helps to understand the soil-plant relationship, as it represents the area of root exploitation, thus microorganisms capable of increasing this area can contribute to improving the uptake of nutrients, water and other resources by plants.

The microbial isolates *Serratia* sp. (BRM32114) and *Azospirillum* sp. (BRM 63574) were the ones that provided greater root volume in corn seedlings (Table 1), with increments of respectively 106.06% and 90.90% compared to the control treatment. The large increase observed in this parameter can be explained by the greater production of phytohormones by the microorganisms involved, proving better results from treatments containing rhizobacteria in root development compared to the control treatment (without microorganisms) (CHAGAS *et al.*, 2015). The *Bacillus* sp. (BRM 63573) also differed statistically from the control treatment, with an increase of 66.66%.

Seedlings treated with *Azospirillum* sp. (BRM 63574) were the ones that presented the best results for the variable of root dry mass, obtaining dry mass 118.69% higher than the control treatment (Table 2). Treatment with *Bacillus sp*. (BRM 63573) also showed a significant increase in dry mass, which was 81.90% higher compared to the control. Bacteria that produce indole-3-acetic acid (IAA) can increase plant growth, especially root initiation and elongation, resulting in greater root mass (SHAO *et al.*, 2015). Hungria (2011), states that the stimulation of root growth by *Azospirillum* is due to the production of auxin, gibberilins and cytokinins, improving the absorption of water and minerals, greater tolerance to stress and resulting in a more vigorous and productive plant.

Table 2. Root dry mass (RDM), shoot dry mass (SDM) and total dry mass (TDM) of corn seedlings, cultivar AG 8088, as a function of treatment with rhizobacteria. Evaluation carried out 14 days after corn sowing.

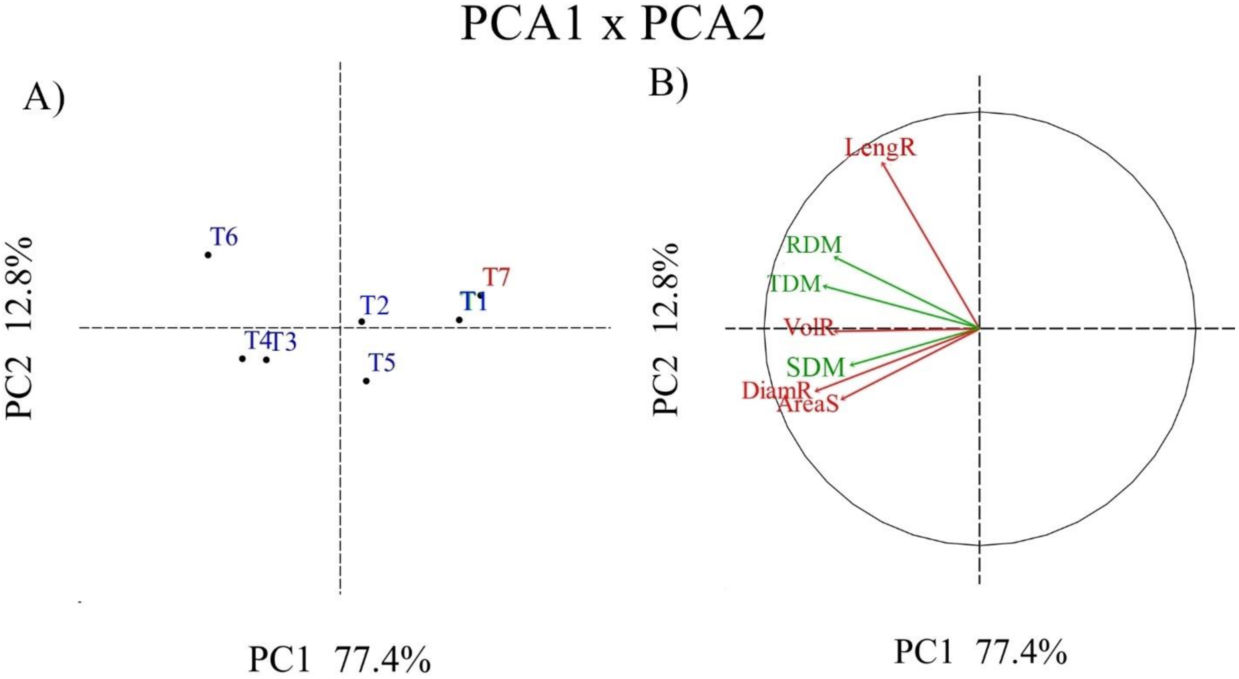
Treatments	**RDM**	**SDM**	**TDM**
	(g)	**(g)**	**(g)**
Serratia marcenses (BRM 32113)	0.0280 c	0.0320 bc	0.0600 c
Burkholderia cepacea (BRM 32111)	0.0362 bc	0.0405 ab	0.0767 bc
Serratia sp. (BRM32114)	0.0491 bc	0.0434 a	0.0925 ab
Bacillus sp. (BRM 63573)	0.0613 ab	0.0433 a	0.1046 a
Azospirillum brasilense (Ab-V5)	0.0414 bc	0.0409 ab	0.0823 bc
Azospirillum sp. (BRM 63574)	0.0737 a	0.0435 a	0.1172 a
Controle	0.0337 c	0.0303 c	0.0640 bc
CV (%)	26.04	17.51	25.31

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

Higher values of shoot dry mass were observed in seedlings treated with *Azospirillum* sp. (BRM 63574), *Serratia* sp. (BRM32114) and *Bacillus* sp. (BRM 63573), with increments of 43.50%, 43.23% and 42.90% respectively each, compared to the control treatment (Table 2). Sousa *et al.* (2018), found that *Azospirillum* sp. and *Bacillus* sp. were able to increase shoot and root dry mass of corn, indicating that a corn seedling with a larger root system can translocate more nutrients to the shoot and increase its vegetative growth. It is noteworthy that seedlings with greater accumulation of dry mass of shoots part signal greater absorption and accumulation of nutrients, which can directly influence their development, greater capacity to resist stress and especially in their productive potential. Regarding the total dry mass of corn seedlings, treatments with *Azospirillum* sp. (BRM 63574) and *Bacillus* sp. (BRM 63573) were the ones that stood out the most, providing increases of 83.13% and 63.44% respectively. Chagas *et al.* (2018) observed that the treatment with *Bacillus* in corn seeds provided higher values of shoot and total dry mass for the seedlings after 20 days of emergence. The results obtained corroborate with Braccini *et al.* (2012), who observed that the inoculation of corn seeds with *Azospirillum* sp. promoted an increase in the dry matter of inoculated plants compared to the other non-inoculated ones.

Regarding the principal component analysis, it can be observed 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 root dry mass of Corn seedlings treated with multifunctional microorganisms were best described by two main components (PCs), accounting for 90.2% of the data variation, that is, PC1 (77.4%), PC2 (12.8%) (Figure 2). 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 2). For example, PC1 was negatively correlated to all analyzed variables. On the other hand, PC2 was positively correlated with total root length, root dry mass and total dry mass, and negatively correlated with root diameter, total surface area, root volume and shoot dry mass.

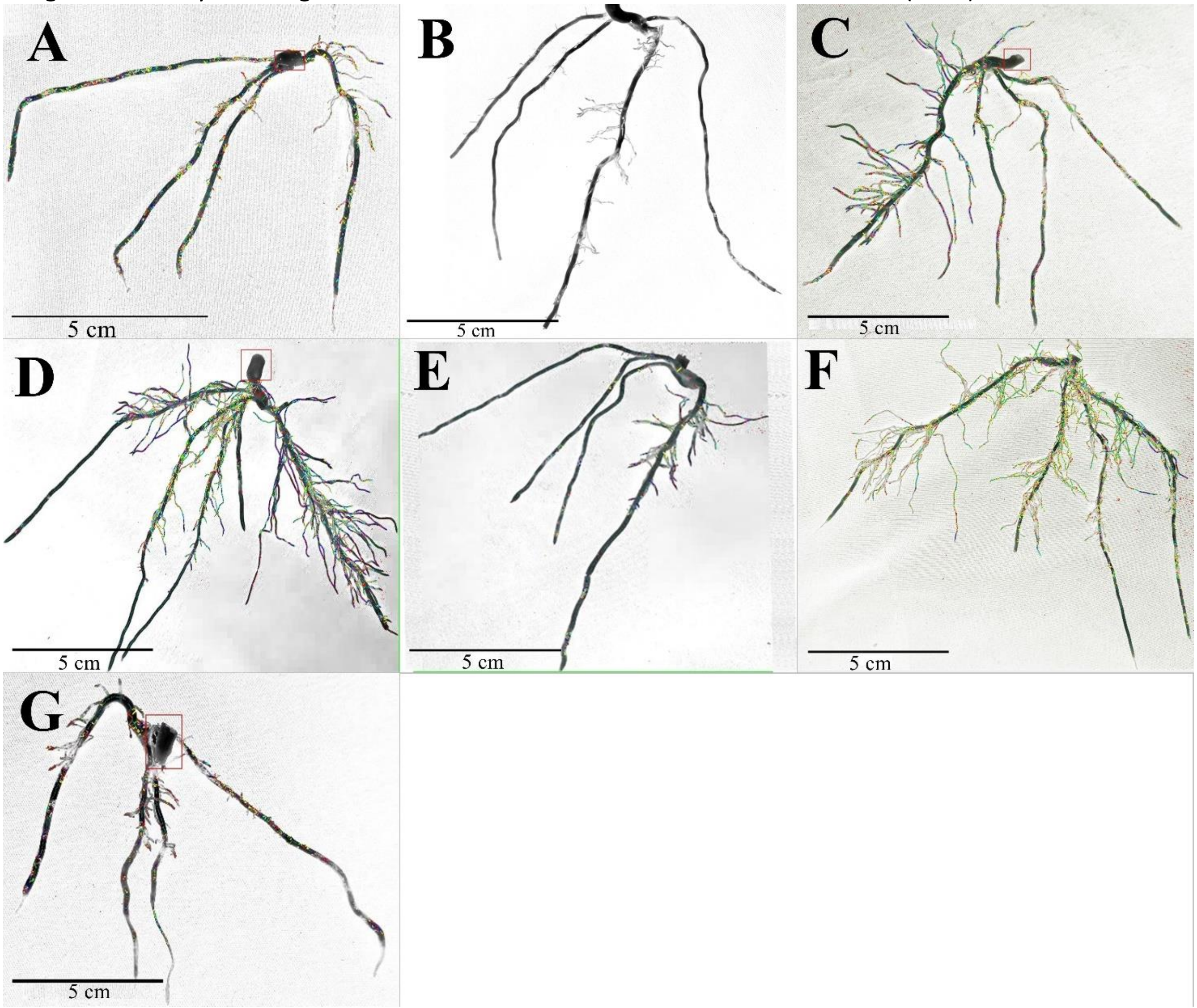
Figure 2. Principal component analysis (PCA) PCA1 X PCA2, explaining the correlations between the evaluated variables and seven treatments with isolates of rhizobacteria and the control (without the microorganism). **A.** Biplot graph for treatments: T1. *Serratia marcenses* (BRM 32113), T2. *Burkholderia cepacea* (BRM 32111), T3. *Serratia* sp. (BRM 32114), T4. *Bacillus* sp. (BRM 63573), T5. *Azospirillum brasilense* (Ab-V5), T6. *Azospirillum* sp. (BRM 63574) and T7. control treatment (without microorganism), and **B.** graph with the variables correlation circle.



Based on the representational quality of treatments with isolated microorganisms and in combination for the analyzed variables, *Serratia* sp. (BRM32114) and *Bacillus* sp. (BRM 63573) obtained the highest positive correlation for root diameter, total root surface area, root volume and shoot dry mass (Figure 2). The variables total root length, root dry mass and total dry mass were positively correlated to the treatment with *Azospirillum* sp. (BRM 63574). Treatments with *Burkholderia cepacea* (BRM 32111), *Serratia marcenses* (BRM 32113), *Azospirillum brasilense* (Ab-V5) and the control treatment did not correlate positively with any of the analyzed variables. The interaction between different species and even lineages of rhizobacteria with plants is not a simple process and there is no consensus on the subject (SILVEIRA; FREITAS, 2007). The promotion of plant growth is directly linked to a specified rhizobacteria-host relationship, in which a specific relationship leads to greater symbiotic action between those involved. Rhizobacteria isolated from different plant species may exhibit specificity in biological growth promotion (ENEBAK *et al.*, 1998; BRUNETTA *et al.*, 2007). The low specificity of these rhizobacteria, initially isolated from upland rice plants, with the corn cultivar used, may explain the little contribution they provided to the initial development of corn.

It is observed that the best results in relation to growth and development were from treatments composed by genera capable of producing phytohormones. This corroborates what was found by Fernandes *et al.* (2021), who also observed increased growth and early root development of upland rice seedlings due to the use of multifunctional microorganisms, citing the production of plant growth hormones as one of the possible reasons for this increased development. Phytohormones act in the meristematic regions of plants, promoting cell growth and elongation, and in the case of roots this can generate greater absorption of water and minerals, resulting in more vigorous and productive plants (NASCENTE *et al.*, 2017). This greater root development was observed in the images of the root system of corn seedlings treated with rhizobacteria compared to control treatment (Figure 3).

Figure 3. Root system of corn seedlings subjected to microbiolization with multifunctional microorganisms: **A.** *Serratia marcenses* (BRM 32113), **B.** *Burkholderia cepacea* (BRM32111), **C.** *Serratia* sp. (BRM32114), **D.** *Bacillus* sp. (BRM 63573), **E.** *Azospirillum brasilense* (Ab-V5), **F.** *Azospirillum* sp. (BRM 63574), and **G.** Control (no microbiolization). Corn seedlings were photographed 14 days after sowing with a digital camera; Images were analyzed using WhinRHIZO 2012 software. Source: Personal Archive (2021).



Treatment of corn seeds with *Azospirillum* sp. (BRM 63574) presented higher values for the analyzed variables of the seedling root system, except for the total root surface area (Table 1). From the images of the root system of the plants, the best development was observed when the seeds were treated with *Azospirillum* sp. (BRM 63574), which presented a greater volume of lateral and adventitious roots, as well as *Serratia* sp. (BRM32114) and *Bacillus* sp. (BRM 63573) who also showed excellent results compared to the control treatment (Figure 3). According to Milléo and Cristófoli (2017), is an advantage for corn plants the capacity of *Azospirillum* sp. to modify the morphology of the root system, increasing not only the number of roots, but also the average diameter of the lateral and adventitious roots, consequently increasing the volume of explored soil. The production of growth hormones can be considered an important mechanism for the initial growth of plants, since the efficiency of phytohormones that regulate growth varies according to the plant's stage of development, their action being more effective in the emergence of seedlings and in early development (DOURADO NETO *et al.*, 2014). The greater capacity of phytohormones production by the inoculated plants may have been essential for the better performance of

Azospirillum sp. in promoting corn root growth compared to the control treatment.

Conclusions

Corn seedlings treated with the multifunctional microorganisms *Bacillus* sp. (BRM 63573), *Serratia* sp. (BRM32114) and *Azospirillum* sp. (BRM 63574) showed an increase in the root and shoots system compared to untreated seedlings. The isolate *Azospirillum* sp. (BRM 63574) provided higher values in the variables of total length, diameter, volume, root dry mass, shoot dry mass and total dry mass compared to the control treatment. The use of multifunctional microorganisms is a promising alternative for better root and shoots development of corn seedlings.

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