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Use of multifunctional microorganisms in corn crop

Utilização de microrganismos multifuncionais na cultura do milho

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ABSTRACT - In the composition of the soil microbiome, there are numerous microorganisms capable of promoting plant growth, better known as plant growth-promoting microorganisms. The study aimed to determine the effects of multifunctional microorganisms, alone or in combination, on shoot, root, and total biomass production, gas exchange, macronutrient content, yield components, and grain yield of corn plants. The experiment was carried out in a greenhouse in a completely randomized design, with four replications. Twenty-six treatments consisted of isolated or combined microbiolization of corn seeds with the rhizobacteria *Bacillus* sp. (BRM 32109, BRM 32110, and BRM 63573), *Burkholderia cepacea* (BRM 32111), *Pseudomonas* sp. (BRM 32112), *Serratia marcenses* BRM 32113, *Serratia* sp. (BRM 32114), *Azospirillum brasilense* (Ab-V5), and *Azospirillum* sp. (BRM 63574), an isolated of fungus *Trichoderma koningiopsis* (BRM 53736), and a control treatment (without the application of microorganisms). At seven and 21 days, two more applications of the same treatments were carried out in the soil and the plants, respectively. The microorganisms applied alone or in combination promoted significant increases of 49% in corn plant biomass, 30% in gas exchange, 36% in macronutrient content, and 33% in grain yield. Isolates BRM 32114, Ab-V5, BRM 32110, and BRM 32112 and the combinations BRM 32114 + BRM 53736, BRM 63573 + Ab-V5, and BRM 32114 + BRM 32110 promoted better benefits to corn, allowing us to infer that the use of beneficial microorganisms significantly affects the development of corn plants.

Keywords: Rhizobacteria. Fungus. Co-inoculation. Yield. *Zea mays*.

RESUMO - Na composição do microbioma do solo tem-se inúmeros microrganismos, capazes de promover o crescimento vegetal, mais conhecidos como microrganismos promotores de crescimento de plantas. Objetivamos determinar os efeitos de microrganismos multifuncionais, isoladamente ou em combinação, na produção de biomassa da parte aérea, raiz e total, nas trocas gasosas, no teor de macronutrientes, componentes de produção e produtividade de grãos de plantas de milho. O experimento foi conduzido em casa de vegetação em delineamento inteiramente casualizado, com quatro repetições. Utilizou-se 26 tratamentos que consistiram na microbiolização isolada ou combinada das sementes de milho com as rizobactérias BRM 32109, BRM 32110 e BRM 63573 (*Bacillus* sp.), BRM 32111 (*Burkholderia cepacea*), BRM 32112 (*Pseudomonas* sp.), BRM 32113 (*Serratia marcenses*) and BRM 32114 (*Serratia* sp.), Ab-V5 (*Azospirillum brasilense*) e BRM 63574 (*Azospirillum* sp.), um isolado de fungo BRM 53736 (*Trichoderma koningiopsis*) e um tratamento controle (sem a aplicação de microrganismos). Aos sete e 21 dias, foram realizadas mais duas aplicações dos mesmos tratamentos, no solo e nas plantas, respectivamente. Os microrganismos aplicados isoladamente ou em combinação promoveram incrementos significativos de 49% na biomassa das plantas de milho, 30% nas trocas gasosas, 36% no teor de macronutrientes e 33% na produtividade. Os isolados BRM 32114, Ab-V5, BRM 32110 e BRM 32112 e as combinações BRM 32114 + BRM 53736, BRM 63573 + Ab-V5 e 32114 + BRM 32110 promoveram melhores benefícios ao milho, nos permitindo inferir que o uso de microrganismos benéficos afetam significativamente o desenvolvimento das plantas de milho.

Palavras-chave: Rizobactérias. Fungo. Coinoculação. Produtividade. *Zea mays*.

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INTRODUCTION

Corn (*Zea mays* L.) is a species from the Poaceae family, which has C4 metabolism, is well known for producing grains rich in starch, and is widely used and appreciated in human and animal nutrition. World corn production in the last harvest was 1.148 billion tons in an area of 207 million hectares planted (USDA, 2022a). The largest producers in the world are the USA, China, and Brazil, which produced in the 2021/2022 harvest approximately 384 and 116 million tons, respectively (USDA, 2022b).

The conventional production systems of corn are mainly dependent on large amounts of nitrogen fertilizers, in addition to the excessive use of other nutrients and pesticides that generate negative financial and environmental impacts. The exaggerated use of these synthetic products by the conventional system can cause contamination of soil, rivers, food, and animals and poisoning of farmers (LOPES; ALBUQUERQUE, 2018), in addition to increasing production costs. Within these perspectives, an alternative would be the use of bacteria and other microorganisms that inhabit the rhizosphere region close to the root, which act as plant growth promoters based on symbiotic relationships (GOMES et al., 2016). These microorganisms can contribute to better absorption of soil nutrients by plants, in addition to their better development, contributing to a reduction in the need for fertilizers and pesticides.

The rhizobacteria stand out among the groups of microorganisms that play a role in promoting plant growth (SHAIKH et al., 2022). The inoculation of plant growth-promoting rhizobacteria (PGPRs) represents a sustainable alternative for reducing the use of pesticides and fertilizers in plant cultivation (SILVA et al., 2018). This is possible because PGPRs present favorable responses to plants, promoting plant growth through several mechanisms of action such as phosphate solubilization, production of phytohormones and siderophores, and biocontrol of pathogens and pest insects (ARAÚJO; GUABERTO; SILVA, 2012).

The fungi from the *Trichoderma* genus are also among the main growth-promoting microorganisms in plants, as they have very similar mechanisms of action to those of PGPRs. (SILVA et al., 2020). After selection and characterization, greenhouse studies confirmed the potential use of these microorganisms, as significant increases in gas exchange and biomass production of upland rice and irrigated rice plants were observed (NASCENTE et al., 2017).

The multiple mechanisms of action present in PGPRs make the combined use of two or more species in crops of great interest, as they are an excellent alternative for farmers to face the new challenges of modern agriculture, where it is necessary to maintain high yield with the least possible impact on the environment (SANTOS et al., 2020). Agricultural activity can greatly benefit from the use of microbial diversity in new biotechnological processes (GUPTA et al., 2022). These benefits meet the challenge of agricultural activity and involve the successful management of natural resources, satisfying the nutritional requirements of crops, and favoring high yield and quality production that meet the sustainability indexes of the agricultural sector (BLANCO-VARGAS et al., 2020).

We tested the hypothesis that using previously selected microorganisms as plant growth promoters can provide increased photosynthetic rates, dry matter accumulation, and nutrient contents in the shoot and root of corn plants. Thus, the following study aimed to determine the effect of the application of multifunctional microorganisms, isolated or mixed, on the production of shoot and root dry matter, gas exchange, nutrient content in shoot and root, yield components, and grain yield of corn plants under controlled conditions.

MATERIAL AND METHODS

The experiment was conducted under controlled conditions at the Embrapa Rice and Beans in Santo Antônio de Goiás, GO, Brazil. A Latossolo Vermelho Escuro (Ácrico) (SANTOS et al., 2018) was used, taken from the surface layer (0.0-0.2 m) of an area that was for more than 20 years cultivated with *Urochloa brizantha* as pasture. The chemical characteristics of the soil were determined according to the methods described by Claessen (1997): pH in H_2O = 5,5; O.M. = 0,7 g dm^{-3}; P-Mehlich = 6 mg dm^{-3}; K = 0,7 $mmol_c$ dm^{-3}; Ca^{2+} = 9 $mmol_c$ dm^{-3}; Mg^{2+}= 4 $mmol_c$ dm^{-3}; Al^{3+} = 2 $mmol_c$ dm^{-3}; H + Al = 40 $mmol_c$ dm-^{3}; Sum of bases = 14 $mmol_c$ dm^{-3}; Potential CEC (T) = 54 $mmol_c$ dm^{-3}; effective CEC (t) = 16 $mmol_c$ dm^{-3}. Three weeks before corn sowing, 104 pots with a capacity of 10 kg were filled with soil and fertilized with 20 grams of N-P_2O_5-K_2O (5-30-15). Two topdressing fertilizations were carried out, one at 30 days with N-P_2O_5-K_2O (20-00-20) and another at 50 days with urea, seeking to meet the nutritional needs of the crop. During the experiment, all pots were sprinkler irrigated until they reached the soil field capacity.

The experimental design was completely randomized, with 26 treatments and four replications. The treatments consisted of microbiolization of corn seeds, cultivar AG 8088, with different microorganisms and their combinations in pairs, with nine isolates of rhizobacteria: *Bacillus* sp. (BRM 32109, BRM 32110, and BRM 63573), *Burkholderia cepacea* (BRM 32111), *Pseudomonas* sp. (BRM 32112), *Serratia marcenses* (BRM 32113), *Serratia* sp. (BRM 32114), *Azospirillum brasilense* (Ab-V5), and *Azospirillum* sp. (BRM 63574) and *Trichoderma koningiopsis* (fungus isolate - BRM 53736). A control treatment was also included without the application of microorganisms. Additionally, at seven and 21 days, solutions with the microorganisms were applied according to each treatment. The main characteristics of the microorganisms used in the experiment were described (Table 1). These microorganisms isolated from the roots of upland rice plants are identified, stored, and preserved at the Multifunctional Microorganisms collection at Embrapa Rice and Beans.

The treatments were distributed as follows: (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). Before any experiment involving the combination of microorganisms, compatibility tests are carried out at Embrapa Rice and Beans. They are not even tested in greenhouse or field experiments if there is no compatibility. Therefore, there is no incompatibility among the combination of microorganisms tested in this trial.

Table 1. Collection code, geographic origin, biochemical characteristics, and taxonomic classification of the nine rhizobacteria and one fungus isolates used in the present experiment.

Code[A]	Origin[B]	Color[C]	Biochemical[D]					Taxonomic[E]
			AIA[F]	Celul.[G]	Phos[H]	Sider.[I]	Biofilm[J]	
BRM 32109	GO/Brazil	White		+	+	+	+	*Bacillus* sp.
BRM 32110	PA/Brazil	White		+	+		+	*Bacillus sp.*
BRM 32111	PA/Brazil	Yellow		+	+	+	+	*Burkholderia cepacea*
BRM 32112	GO/Brazil	Yellow		+	+	+	+	*Pseudomonas* sp.
BRM 32113	PA/Brazil	Pink	+	+		+	+	*Serratia marscenses*
BRM 32114	PA/Brazil	Pink	+	+	+	+	+	*Serratia* sp.
BRM 63574		White	+	+	+	+	+	*Azospirillum sp.*
BRM 63573		White	+	+	+	+	+	*Bacillus* sp.
Ab-V5	PR/Brazil	Yellow	+	+	+	+	+	*A. brasiliense*
BRM 53736		Green	+		+			*Trichoderma koningiopsis*

[A]Number code of rhizobacterial isolates in the Microorganisms and fungi Multifunction Embrapa Rice and Beans collection; [B]Geographical origin of each isolate; [C] Colony color, [D] Biochemical characterization and [E] Taxonomic classification of each isolate, described by Nascente et al. (2017); [F] Acid idol acetic producer; [G] cellulase producer; [H] Phosphatase producer; [I] siderophores producer; [J] biofilm producer.

Bacterial suspensions for seed microbiolization and applications were prepared based on water from cultures grown for 24 hours in solid medium 523 (KADO; HESKETT, 1970) at 28 °C and with the concentration fixed at 10^{8} CFU (colony forming unit) by adjusting the optical density reading of the cell suspension to 0.5, using a spectrophotometer at a wavelength of 540 nm. Depending on the treatment, the corn seeds were immersed in each suspension of microorganisms, and control seeds were immersed in distilled and autoclaved water. Microbiolization was carried out for 4 hours under constant agitation at 25 °C. For the production of combinations of microorganisms, equivalent amounts of each microorganism involved in the microbiolization solution were added.

Before the microbiolization process, the seeds were disinfected by immersion in 70% alcohol for two minutes and then in 0.05% sodium hypochlorite for the same period, followed by drying at room temperature (29 °C). The microbiolization methodology used for corn seeds was an adaptation of the microbiolization of rice seeds proposed by Filippi et al. (2011). Before performing the microbiolization of corn seeds for planting the experiment, a test was carried out to determine the ideal period for the seeds and contact with the bacterial suspension. Corn seeds (cultivar AG 8088) were submerged in a suspension basis of water from cultures with isolate Ab-V5 (*Azospirillum brasilense*) for four different periods (2, 4, 6, and 16 hours) and also in deionized water (control treatment) for the same periods, totaling eight treatments and four repetitions. After each period of microbiolization, the seeds were removed from the suspension, dried at room temperature, and sown in 500 ml cups containing soil. After 15 days, the best development and growth of the plants were observed in each treatment. The best results were observed with 4 hours of microbiolization, considering the achievement of higher values of root and shoot mass of the seedlings compared to the other periods.

For *T. koningiopsis* (BRM 53736), the isolate was cultivated in an Erlenmeyer flask containing parboiled rice as substrate (SILVA et al., 2011). The substrate was prepared with 25 g of rice and 20 mL of distilled water in a 125 mL Erlenmeyer flask. Seven rice grains were added to the substrate with T. koningiopsis spores from inoculums available at the Laboratory of Agricultural Microbiology of Embrapa Rice and Beans. They grew for seven days in B.O.D. at 28°C with a 12-hour daily photoperiod. After inoculation, the flask was shaken daily to homogenize and facilitate the fungus colonization. Afterward, the rice grains were milled in a blender at minimum speed to release the fungal spores. For seed treatment, solutions were produced with concentrations of 10 g of *T. koningiopsis* diluted in one liter of distilled water, where later the concentration was fixed in 10^{8} conidia ml^{-1} (SILVA et al., 2011).

For microbiolization, a solution was used at a dose of 250 mL 50 kg^{-1} of seeds. Six corn seeds were sown per pot. Ten days after planting, the thinning was carried out, keeping only two plants per pot. According to each treatment, two applications of suspensions with microorganisms or combinations of microorganisms were made, the first with 100 mL of suspension applied directly to the soil seven days after sowing and the second with a foliar spray of 30 mL of suspension at 21 days after sowing. The crop management was carried out following the recommendations and needs of the crop, and irrigation was done manually daily.

The evaluations carried out to determine the effects of microorganisms applied to the corn crop during the conduct of the experiment were:

Gas exchanges: The gas exchange assessments were determined using a portable IRGA infrared gas meter

(LCpro+, ADC BioScientific), between 08:00 and 10:00 a.m., at flowering (reproductive period). One corn plant per pot was chosen, totaling four plants evaluated per treatment, using the flag leaf for evaluation. In corn plants, the following gas exchange evaluations were carried out: photosynthetic rate (A - μmol CO_2 m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), and leaf temperature (Tleaf - °C).

Biomass production: When the corn stalks reached the harvest stage, the shoot of the vases were cut and placed in kraft paper bags, properly identified. Three days after the shoot collection, the soil of each pot was washed and sieved to separate the roots, then placed in kraft paper bags and properly identified. Shoot and roots were dried in an air-forced circulation oven, at 65 ºC, until constant mass. Then, the material in each bag was weighed to determine the shoot and root dry biomass and total dry biomass (shoot + root).

Nutrient content in corn plants: Representative samples were taken from the collected plants and roots, after drying and weighing, which were ground using a mechanical mill, and sent to the Agroenvironmental Analysis Laboratory of Embrapa Rice and Beans for the analysis and determination of nutrient contents (N, P, and K) in the shoot and root, following the recommendations ok Malavolta, Vitti and Oliveira (1997).

Grain yield: The experiment was harvested when the corn plants reached physiological maturity. Thus, cob diameter, cob length, and cob mass were measured. The grains went through a drying process, and the moisture values were corrected to 13% (dry base). After this drying, the total mass of grains produced per pot (yield - per pot) and the 100-grain weight was measured using a digital scale and a grain counter.

The data were subjected to analysis of variance and means grouped by the Scott-Knott test ($\alpha \leq 0.05$). The SAS statistical package was used (SAS, 1999). A principal component analysis (PCAs) was performed to describe the correlations between the response variables and the microorganisms alone and in combination. The principal components (PCs) were loaded with response variables when the correlation test produced $r \geq 0.50$. The first three PCs were responsible for 68.6% of the data variation. The biplot (two-dimensional graph) correlated the response variables and the microorganisms granted, and in combination, it was used in the statistical software R, built with the "FactoExtra" package (KASSAMBARA, 2015).

RESULTS AND DISCUSSION

According to the analysis of variance, there were statistically significant differences between treatments for gas exchange, shoot, root, and total dry biomass, macronutrient content in the root and shoot, as well as for the grain yield of corn plants treated with multifunctional microorganisms, in isolated or combined use (Figures 1 to 5). The plants treated with the BRM 32113 isolate had the lowest photosynthetic rates, being even statistically close to the control treatment (Figure 1A). Treatment with Ab-V5 showed the best results for the photosynthetic rate, significantly higher than the control treatment. Nascente et al. (2017) found that growth-promoting rhizobacteria are capable of generating significant increases in the physiological characteristics of plants, such as the photosynthetic rate. This improvement in the physiological attributes of plants is because these rhizobacteria exert beneficial effects on plants through different mechanisms of action, which can be direct such as biological nitrogen fixation, phosphate solubilization, production of phytohormones, or indirect, such as the production of siderophores and biofilm (SINGH; SINGH; PAL, 2017; REZENDE et al., 2021a). The benefits that multifunctional microorganisms can offer to plants can directly or indirectly influence various physiological processes of plants.

The microorganism BRM 32111 was the treatment that promoted the greatest increases in the transpiration rate (E), followed by BRM 32110, BRM 32111, BRM 32114, BRM 63574 + BRM 32110, and BRM 32114 + BRM 53736, which all differed statistically from the control treatment (Figure 1B). Transpiration is an extremely important process for plants, as it generates an interior movement of water crucial for plant growth; thus, its rate must be kept within the optimal limits for each species (BONETTI; FINK, 2020). The use of multifunctional microorganisms can be important to improve the gas exchange of plants, including the transpiration rate, and can be very advantageous for their development (SILVA et al., 2020).

Higher stomatal conductance (gs) values were observed for corn plants treated with BRM 32114 and BRM 32110, which were also statistically different from the control treatment (Figure 1C). Rhizobacterial inoculation can improve seedling growth, resulting in better control of electrolyte leakage, stomatal conductance, and plant water potential (AZIZI et al., 2021). Concerning leaf temperature (Tleaf °C), there were no significant differences between the studied treatments and the control treatment (Figure 1D). Multifunctional microorganisms can have minimal effects on some gas exchange variables; however, they can significantly affect other variables related to grain yield (REZENDE et al., 2021b).

The corn plants treated with BRM 63573 + Ab-V5 were the only ones that showed a significant increase in shoot dry biomass compared to the control treatment (Figure 2A). The combination BRM 63573 + Ab-V5 promoted a 30.76% increase in shoot dry biomass. Dartora et al. (2013) observed increases in shoot dry matter of corn hybrids with *A. brasilense* inoculation compared to non-inoculated plants due to the ability of these bacteria to carry out biological nitrogen fixation and phytohormones production. Chagas et al. (2017) found an increase in the dry mass values of corn plants inoculated with *Bacillus* sp. compared to uninoculated plants; this increase is mainly related to the capacity to produce growth-promoting substances and phosphate solubilization of this genus of rhizobacteria. The treatments with BRM 32112 and BRM 32114 also stood out compared to the control treatment.

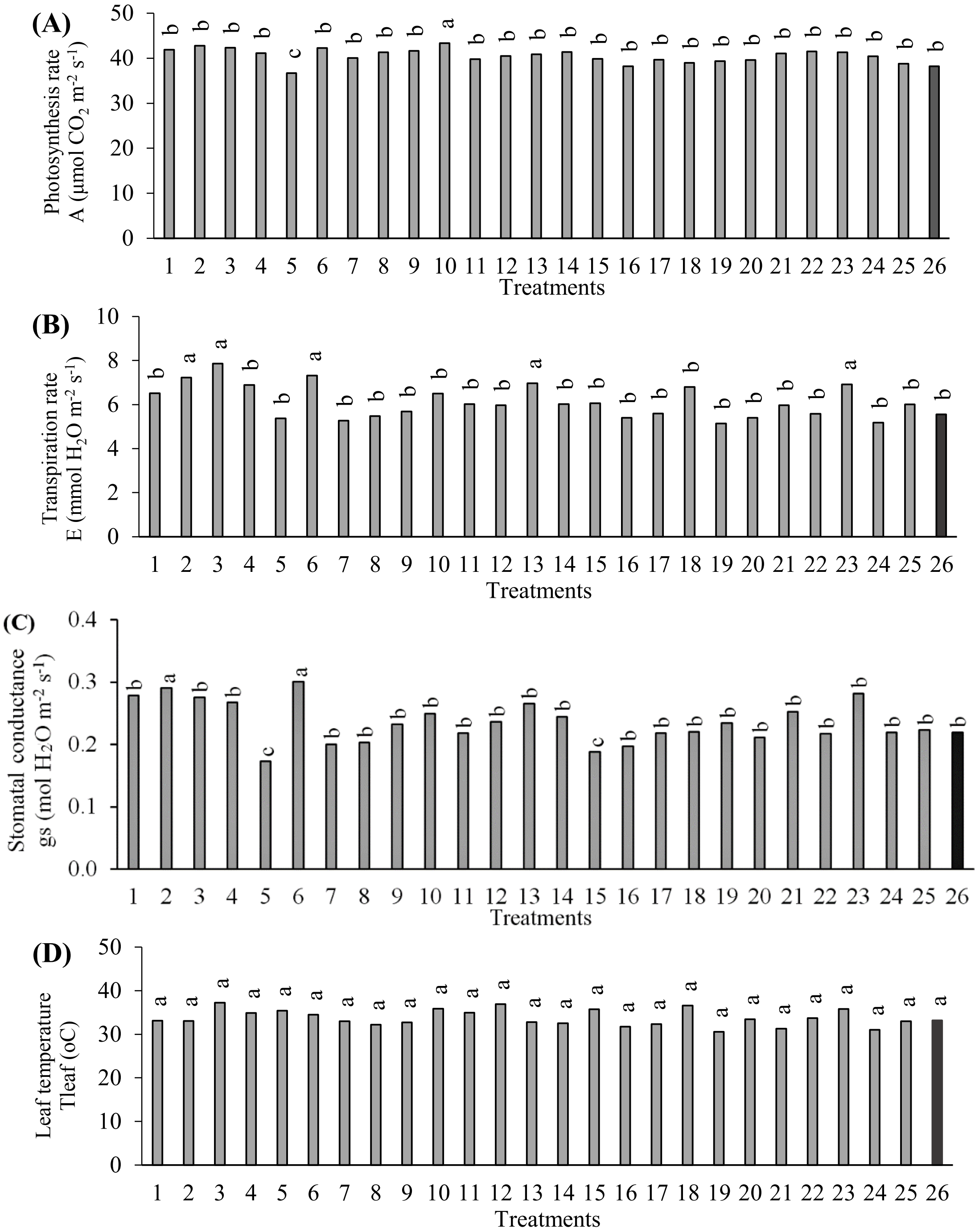

Figure 1. (A) Photosynthesis rate (CV: 8.15%), (B) Transpiratory rate (CV: 16.40%), (C) Stomatal conductance (CV: 26.21%), and (D) Leaf temperature (CV: 11.89%) of treated corn plants with multifunctional microorganisms, in isolated use or combined. *Bars followed by the same letter belong to the same group by the Scott-Knott test. **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 (without microorganisms).

There were higher values concerning root dry biomass for the combinations BRM 63574 + BRM 63573, BRM 32114 + BRM 53736, and BRM 63574 + BRM 32114, which were statistically superior to non-inoculated plants (Figure 2B). Costa et al. (2015) found that inoculation of *Azospirillum* in corn seeds combined with nitrogen fertilization can increase the dry mass of the stalk and root of plants. The genus *Serratia* (BRM 32114), present in two of the combinations, is mentioned in the literature as capable of acting in the production of growth phytohormones (KUMAR et al., 2021) and phosphate solubilization (BLANCO-VARGAS et al., 2020), which are mechanisms that help a lot in the growth and development of roots. Rhizobacterial inoculation can generate effects on root morphology, with the greatest of these effects represented by increased proliferation of root hairs (LIMA et al., 2021), which leads to better soil exploration, in addition to better plant development due to greater capacity for absorption of water and nutrients.

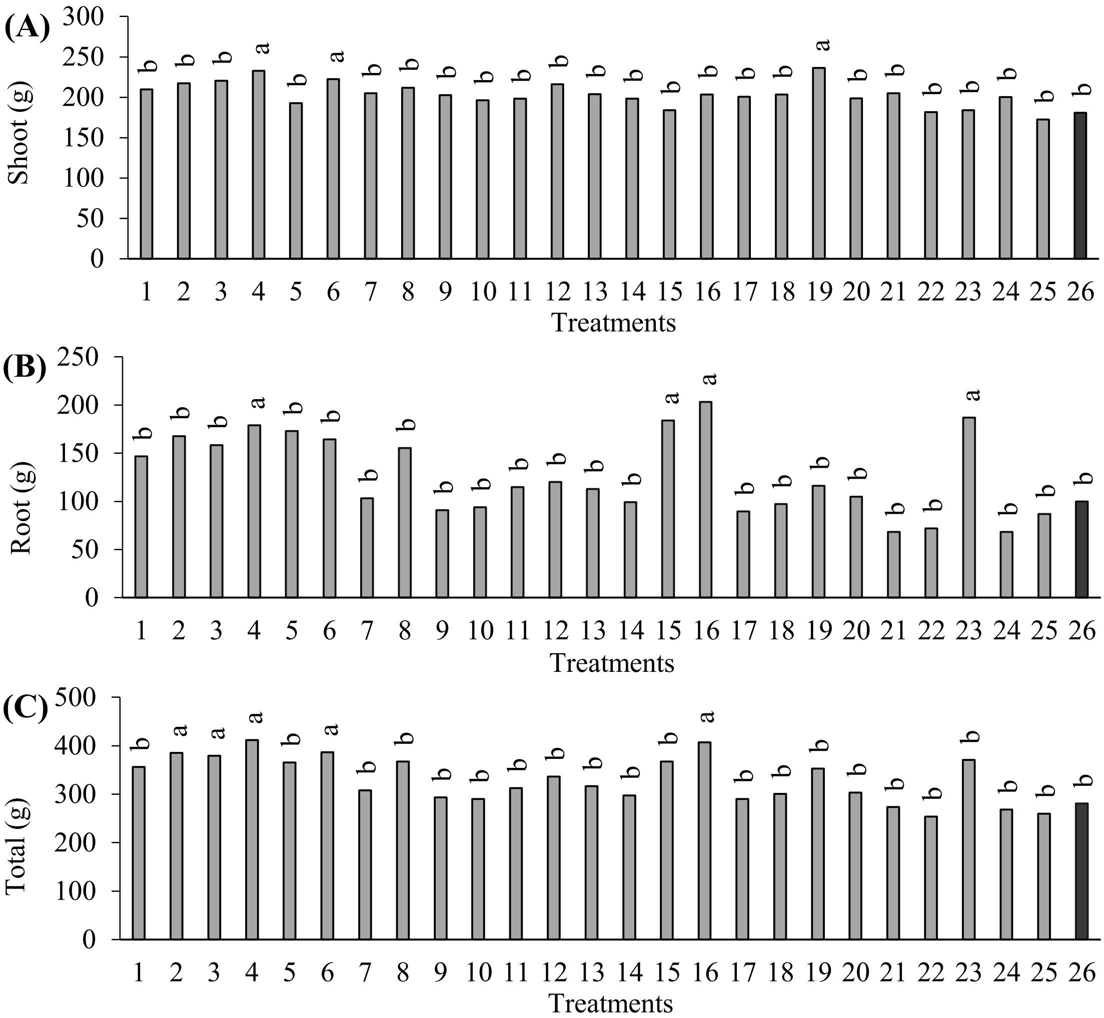

Figure 2. (A) Shoot biomass (CV: 14.44%), (B) Root (CV: 25.68%), and (C) Total (CV: 22.40%) of corn plants treated with multifunctional microorganisms in isolated use or combined. *Bars followed by the same letter belong to the same group by the Scott-Knott test. **Treatments: (1) BRM 32109; (2) BRM 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 (without microorganisms).

In the production of total dry biomass, the highlights were BRM 32112, BRM 63574 + BRM 63573, BRM 32110, BRM 32111, and BRM 32114, all statistically superior to the control treatment (Figure 2C). This stimulus to plant development can be provided by different forms of action of these rhizobacteria, such as the increase in the availability of nutrients, especially phosphorus, caused by the solubilization of forms of P with low solubility; by biological nitrogen fixation; phytohormone production, and pathogen control (MANRIQUE et al., 2019). The worst results concerning total dry biomass were observed in treatments with isolate BRM 53736 and its combinations, noting significant values for both shoot and root dry biomass (Figures 3A e 3B). Both *Trichoderma* and rhizobacteria can have several benefits for plant development, but little is known about the effects of combining the fungus with bacteria (SILVA et al., 2020).

The plants treated with isolates BRM 63574 and Ab-V5 together with the combinations BRM 63574 + BRM 32114, BRM 63574 + Ab-V5, and Ab-V5 + BRM 53736 were the treatments that presented the highest N contents in the plant, adding roots and shoot part, being statistically superior to the control treatment (Figure 3A). The mean increase in the N content of these treatments compared to the control treatment was 20.97%. This increase can be explained by the presence of the genus *Azospirillum* sp. (Ab-V5 and BRM 63574) in all highlighted treatments, which can perform biological fixation and promote better nitrogen absorption in different plant species (REIS et al., 2022; RODRIGUES et al., 2022).

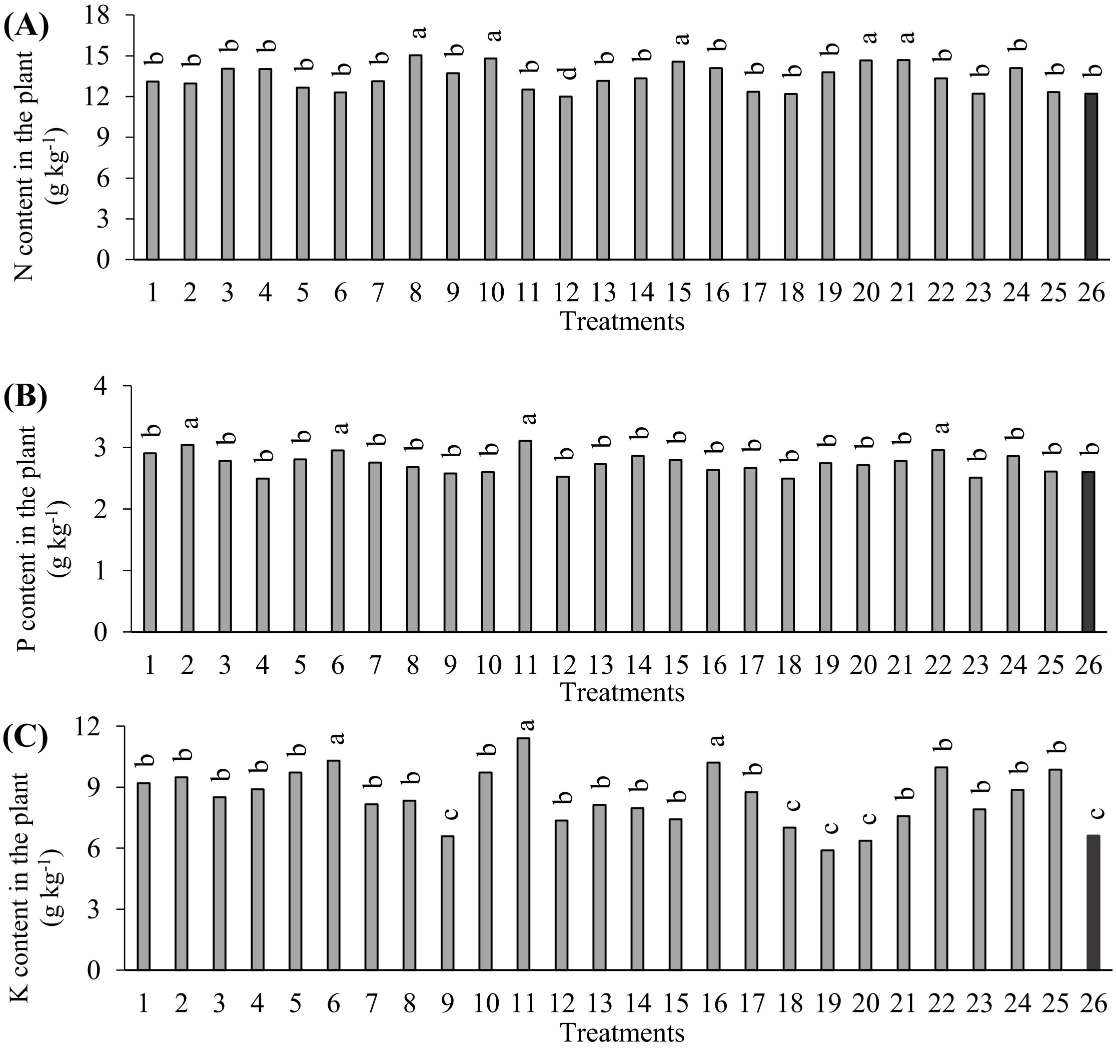

Figure 3. (A) Nitrogen (N) (CV: 14.44%), (B) phosphorus (P) (CV: 12.64%), and (C) potassium (K) (CV: 28.44%) contents of corn plants treated with multifunctional microorganisms in isolated use or combined. *Bars followed by the same letter belong to the same group by the Scott-Knott test. **Treatments: (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 (without microorganisms).

The content of macronutrient P was significantly higher for corn plants treated with the combinations BRM 32114 + BRM 32110, BRM 32110 + BRM 53736, and the isolates BRM 32110 and BRM 32114, compared to the control treatment (Figure 3B). Compared to the control treatment, these treatments showed an average increase of

15.87% in their P content. The genera *Trichoderma* sp., *Bacillus* sp., and *Serratia* sp. are known for their ability to solubilize phosphates (AWAIS et al., 2017; PRASAD et al., 2021). Only the combination BRM 32114 + BRM 32110 was statistically superior to the control treatment (Figure 3C), with an increase of 4.04 g kg^{-1}. Potassium (K) is the second most absorbed nutrient by plants, essential in activating several enzymes that act in the processes of photosynthesis and respiration (PARENTE et al., 2016). It can be inferred then that the application of multifunctional microorganisms positively affected the absorption of nutrients by corn plants through their various mechanisms of action.

The isolates BRM 32114, BRM 32110, BRM 32114 + Ab-V5, and BRM 32114 + BRM 53736 provided higher averages of cob diameter, being statistically superior to the control treatment (Figure 4A). Regarding cob length, only the combination BRM 32114 + BRM 53736 provided statistical superiority compared to plants in which there was no application of multifunctional microorganisms (Figure 4B). The treatments BRM 32114, BRM 32110, and BRM 32114 + BRM 53736 had the highest averages for cob masses, surpassing the control treatment (Figure 4C). The supply of nutrients, mainly N-P-K, has positive effects on corn cultivation, promoting an increase in the length, diameter, and mass of the cob (FREIRE et al., 2010; ROLIM et al., 2018). In this way, multifunctional microorganisms can remarkably produce more vigorous cob, mainly due to their ability to promote greater availability of nutrients to plants, mainly by biological N fixation and nutrient solubilization, as in the case of phosphorus.

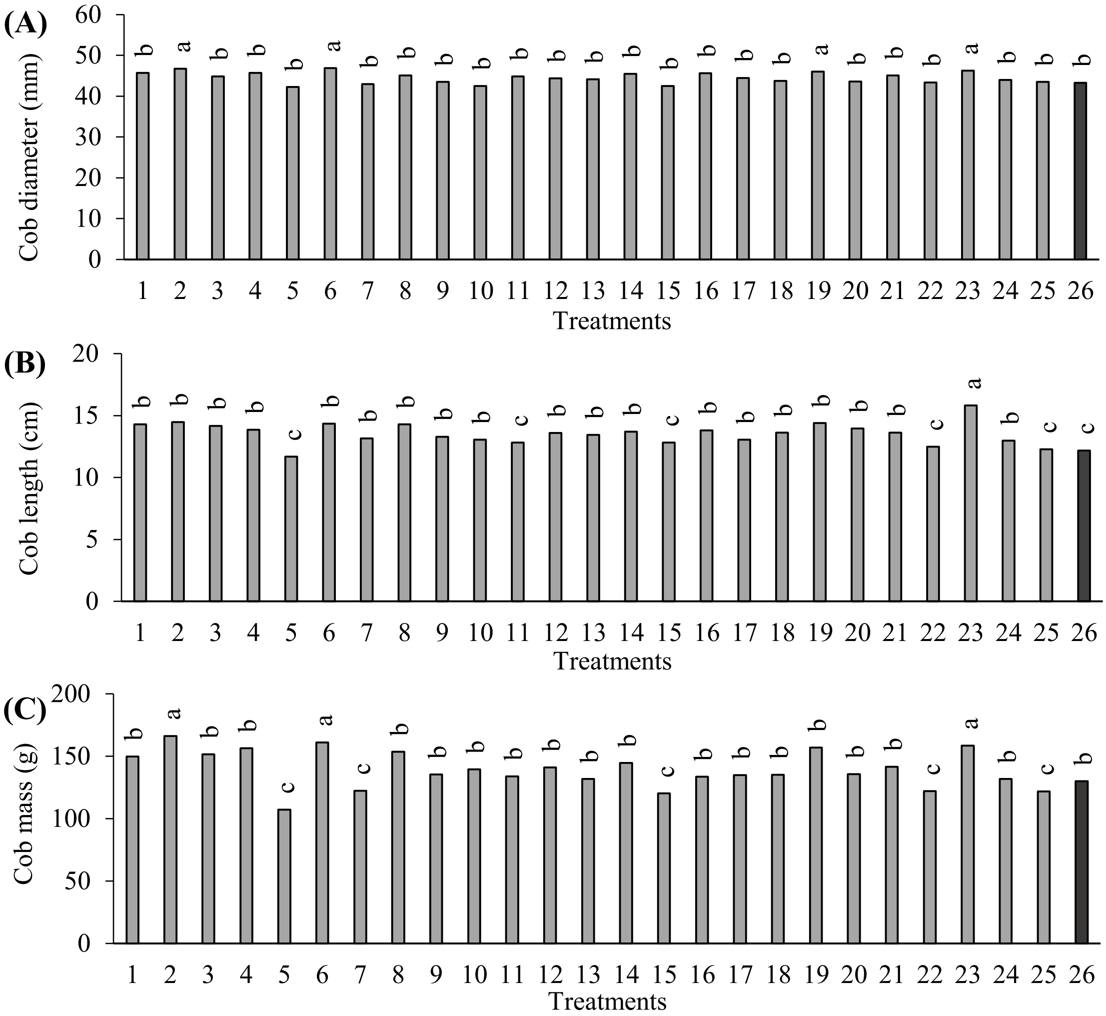

Figure 4. (A) Diameter (CV: 5.16%), (B) length (CV: 13.29%), and (C) cob Mass (CV: 18.49%) of corn plants treated with multifunctional microorganisms in isolated use or combined. *Bars followed by the same letter belong to the same group by the Scott-Knott test. **Treatments: (1) BRM 32109; (2) BRM 32110; (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 (without microorganisms).

Most of the evaluated treatments obtained statistically superior results than the control treatment for 100-grain weight, except for the treatments BRM 32113, BRM 63574 + BRM 32110, BRM 63573 + Ab-V5, BRM 63573 + BRM 53736, and BRM 63574 + BRM 53736 (Figure 5A). On the other hand, the best results for 100-grain weight were observed for the treatments BRM 32114, Ab-V5, and BRM 32110 + BRM 53736. According to Hashem et al. (2019), works with multifunctional microorganisms have contributed to an increase in the growth and/or yield of corn, indicating that the possibility of combined use alone or of two or more rhizobacterial species associated or not with chemical fertilizers can constitute a promising biotechnological alternative for purposes of vegetable production, including corn cultivation.

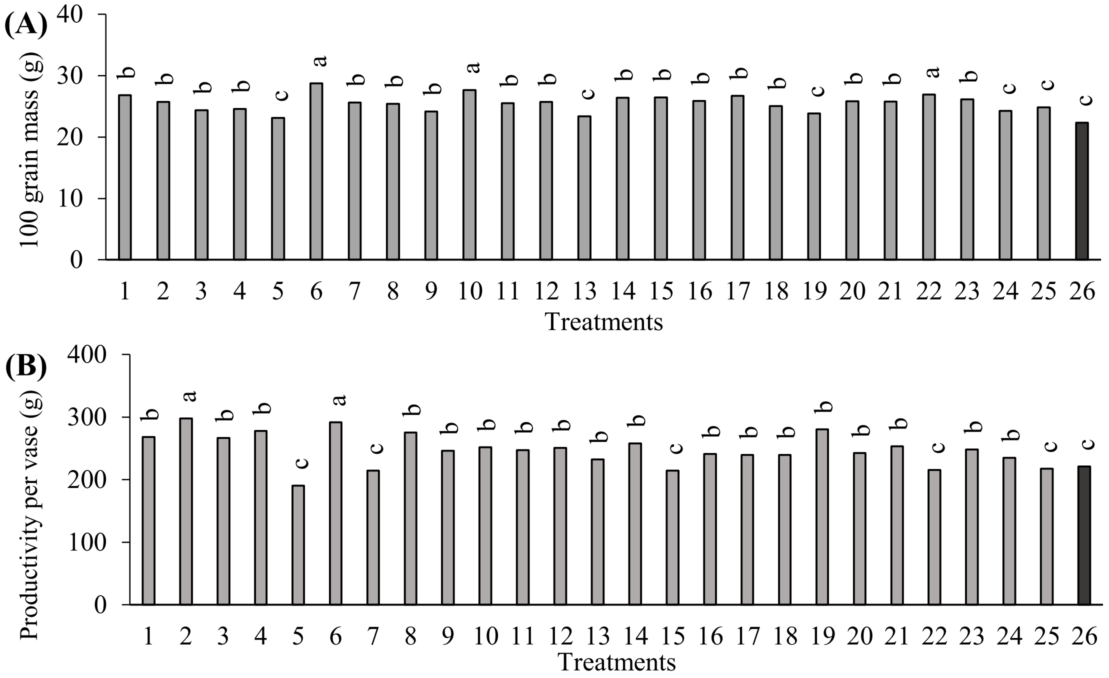

Figure 5. (A) 100-grain weight and (B) grain yield (per pot) of corn plants treated with multifunctional microorganisms in isolated use or combined. *Bars followed by the same letter belong to the same group by the Scott-Knott test. **Treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (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 (without microorganisms).

The BRM 32110 and BRM 32114 isolates provided the highest corn grain yield, significantly higher than the control treatment (Figure 5B). The same treatments promoted increases in the macronutrient content of corn plants, with emphasis on P and K (Figure 3). According to Rezende et al. (2021b), the greater development of roots provided by multifunctional microorganisms improves soil structure. It may increase the capacity of plants to absorb water and nutrients, favoring development, filling, and grain yield. This may explain the satisfactory results obtained, where the better use of macronutrients available in the soil may have provided better plant nutrition and, consequently, greater grain yield.

Regarding the principal component analysis, we could see that the variability of treatments with isolated and combined microorganisms in gas exchange (photosynthesis, transpiration rate, stomatal conductance, and leaf temperature), biomass production (shoot, root, and total), accumulation of plant nutrients (N, P, and K contents), yield components (cob length, cob diameter, cob mass, and 100-grain weight) and grain yield of corn plants treated with multifunctional microorganisms were best described by three main components (PCs), accounting for 68.6% of the data variation, that is, PC1 (40.70%), PC2 (14.9%), and PC3 (13%) (Figure 6).

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 6). For example, PC1 was positively correlated with nitrogen (N), phosphorus (P), potassium (K), 100-grain weight, and leaf temperature. Still, it was negatively correlated with the shoot, root, and total biomass, photosynthesis, transpiration rate, stomatal conductance, cob length, cob diameter, cob mass, and grain yield.

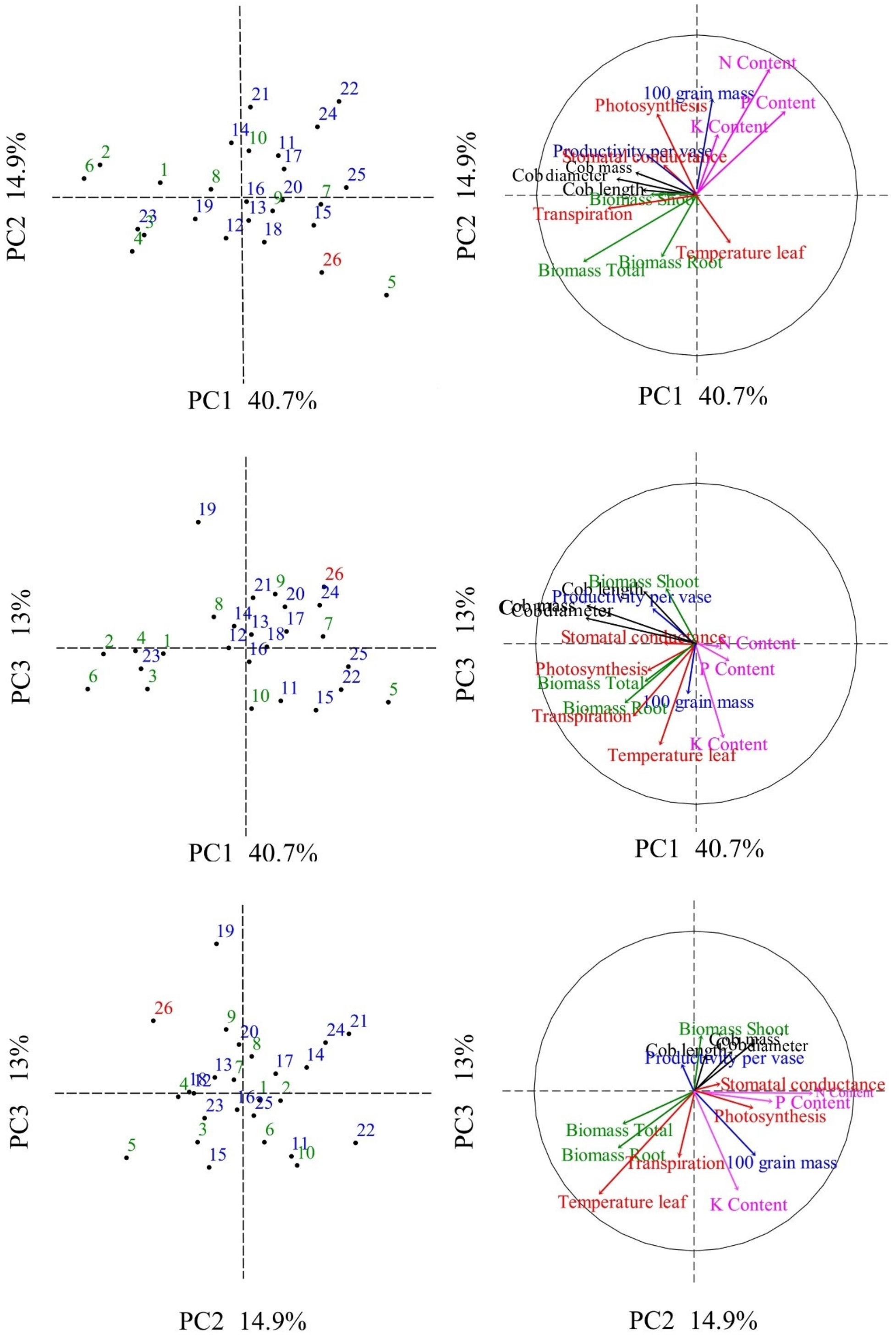

Figure 6. Principal Component Analysis (PCA) with the correlations between the variables and the 26 treatments with isolated and combined microorganisms. Three main components (PCs) accounted for 68.6% of data variation. **Treatments: (1) BRM 32109; (2) BRM 32110; (3) BRM 32111; (4) BRM 32112; (5) BRM 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 (without microorganisms).

On the other hand, PC2 was positively correlated with photosynthesis, transpiration rate, stomatal conductance, N content, P content and K content, cob length, cob diameter, cob mass, 100-grain yield, and grain yield, and negatively correlated with shoot and root dry biomass and leaf temperature. While PC3 was positively correlated with stomatal conductance, shoot biomass, cob length, cob diameter, cob mass, and grain yield and was negatively correlated with photosynthesis, transpiration rate, leaf temperature, root and total biomass, N content, P content, K content, and 100-grain weight.

Based on the representational quality of treatments with microorganisms isolated and combined for the analyzed variables, treatments 3 (BRM 32111), 4 (BRM 32112), and 23 (BRM 32114 + BRM 53736) had the highest positive correlation for transpiration, shoot, root and total biomass (Figure 6). The variables photosynthesis, stomatal conductance, cob length, cob diameter, cob mass, and grain yield were positively correlated with isolates 1 (BRM 32109), 2 (BRM 32110), 6 (BRM 32114), and 8 (BRM 63574). 100-grain weight was positively correlated to treatment 21 (Ab-V5 + BRM 53736), while K content was equally correlated to treatments 10 (Ab-V5) and 14 (BRM 63573 + BRM 32114). The contents of N and P were positively correlated with combinations 22 (BRM 32110 + BRM 53736) and 24 (BRM 63573 + BRM 53736). Finally, leaf temperature was positively correlated to treatment 26.

The results of the present study demonstrate a promising potential for the use of multifunctional microorganisms in promoting benefits from the physiological point of view and grain yield and absorption of nutrients by corn plants. The studies in this direction have great global appeal, mainly because it is a technology of low investment cost, easy to apply and use, non-polluting, and inserted in a desirable context for the sustainable intensification of modern agriculture (SILVA et al., 2020). The use of the genera *Serratia*, *Azospirillum*, *Bacillus,* and *Pseudomonas*, in isolation or combined, stood out for providing beneficial effects to corn, generating positive effects for the corn crop. Although the *Trichoderma* genus did not show good overall results in isolation, its combination with the *Serratia* sp. genus (BRM 32114 + BRM 53736) showed promising results. Future field research may be important to confirm the results observed in a controlled environment.

CONCLUSIONS

The use of beneficial microorganisms provides significant increases in gas exchange, shoot and root biomass, N, P, and K contents and also has direct effects on the yield components of corn. Beneficial microorganisms *Serratia* sp. (BRM 32114), *Azospirillum brasilense* (Ab-V5), *Bacillus* sp. (BRM 32110), and *Pseudomonas* sp. (BRM 32112), together with the combinations BRM 32114 + BRM 53736, BRM 63573 + Ab-V5 and BRM 32114 + BRM 32110 stood out for providing beneficial effects to corn. Therefore, it can be stated that using beneficial microorganisms in corn plants is an interesting sustainable alternative for increasing crop yield.

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