

Full Length Research Paper

Beneficial microorganisms as affecting root development of upland rice

Adriano Stephan Nascente^{1*}, Ishola Zainab Temitope², Marta Cristina Corsi de Filippi¹ and Dennis Ricardo Cabral Cruz²

¹Embrapa Arroz e Feijão, Santo Antônio de Goiás, Goiás, Brazil.

²Escola de Agronomia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil.

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Multifunctional microorganisms can significantly affect root and shoot development of upland rice seedling that could provide an increase in the crop grain yield. The objective of this study was to determine the effect of single and combined microorganism on the root and shoot development of upland rice seedlings. The experiment was arranged in a completely randomized design with treatments consisting of the upland rice seeds treated with single and combined multifunctional microorganisms (M01 (*Serratia marcescens*), M02 (*Bacillus toyonensis*), M03 (*Phanerochaete australis*), M04 (*Trichoderma koningiopsis*), M05 (*Azospirillum brasilense*), M06 (*Azospirillum* species), M07 (*Bacillus* species), M08 to M28 (combination of these microorganisms) and M29 (control – no microorganism). *S. marcescens* with *B. toyonensis* led to the greatest increase (296%) in root length relative to the control. *B. toyonensis* with *A. brasilense* greatly increased root surface area by 209% in comparison to the control. An increased root diameter by 36% was recorded for upland rice inoculated with *A. brasilense* with *Bacillus* spp. in relation to the control. *P. australis* with *Bacillus* spp. greatly increased root volume (47%) in comparison to the control. It can be concluded that multifunctional microorganisms enhanced root length, root surface area, root diameter and volume, and provided better root development.

Key words: Microbiolization, root length, germination, beneficial bacteria, beneficial fungi.

INTRODUCTION

The application of multifunctional microorganisms improves plant development through direct and indirect mechanisms, and has shown that it is possible to make crop management practices more environmentally sustainable (Cruz et al., 2023; Silva et al., 2023). These mechanisms are a result of production of specific metabolites, such as growth stimulants (phytohormones), hydrolytic enzymes, siderophores, antibiotics, and carbon

and nitrogen hence acting as a growth promoter (Silva et al., 2022; Rezende et al., 2021). The multifunctional microorganisms also ensure that the plant receives solubilized nutrients which are fixed in soil minerals and not available to the plants (Sousa et al., 2021). The study of these microorganisms is now currently intense due to the elevating demand for sustainable technologies, which reduces cost of production, increases the productivity,

*Corresponding author. E-mail: adriano.nascente@embrapa.br. Tel: 00 55 62 35332121.

profitability of agribusiness and increases efficiency of fertilizer usage (Couto de Araujo et al., 2021).

The root system is the main organ for plants to absorb nutrients and water, and the root growth condition directly affects the growth of plant shoots (White, 2009; Fernandes et al., 2021). Plant roots can adapt to the changes of soil nutrient conditions through active physiological regulation to absorb more nutrients (Sun et al., 2020). The rhizosphere is the center of the interaction among plant roots, soil, microorganisms, and environment. It is a nutrient-rich habitat and harbors a huge variety of bacteria and fungi that each can have neutral, beneficial or deleterious effects on the plant (Berendsen et al., 2012). The beneficial effect of rhizosphere include effect on plant growth by response to root exudates in the root microorganism environment and carbon sources for the microbial growth activities and the community composition through the process of rhizosphere deposition. The plant will establish beneficial connections with some microorganisms in the rhizosphere (Toju et al., 2018) thereby leading to a positive impact on plant health and productivity.

In an experiment to determine the effect of six multifunctional microorganisms on upland rice seedling performance, Fernandes et al. (2021) found that upland rice seedlings treated with multifunctional microorganisms showed increments in all parameters evaluated in the root system. Furthermore, they stated that microorganism *Azospirillum* species provided higher values in the parameters of total root length, root volume, and dry root mass in upland rice seedlings compared to the control treatment. However, it is imperative to carry out research on the sole and combined effect of these multifunctional microorganisms on the root development of upland rice. Additionally, there is need to evaluate the probable response of other root development parameters (root diameter, root surface area) to multifunctional microorganisms, hence the need for this study. The objective of this study was to determine the effect of single and combined microorganism on the root and shoot development of upland rice seedlings.

MATERIALS AND METHODS

Site description

The experiment was conducted in the Agricultural Microbiology Laboratory at Capivara Farm, headquarters of Embrapa Rice and Beans, located in the Santo Antônio de Goiás, Goiás, Brazil in January 2022.

Experimental design and treatments

The experimental design was a completely randomized with twenty-nine (29) treatments and ten replications. The treatments consisted of the microbialization of the seeds (a process that puts the seeds in contact with the microorganism solution to be tested) of upland rice cultivar BRS A501 CL with the multifunctional microorganisms

Azospirillum spp. (BRM 63574), *Azospirillum brasilense* (AbV5), *Phanerochaete australis* (BRM 62389), *Serratia marcescens* (BRM 32114), *Bacillus toyonensis* (BRM 32110), *Bacillus* species (BRM 63573), *Trichoderma koningiopsis* (BRM 53736) and the control treatment (without microorganisms), then the combination of two microorganisms were also used. The multifunctional microorganisms used in this experiment were deposited in the collection of microorganisms of Agricultural importance of Embrapa Rice and Beans.

Preparing suspension of microorganism

To generate the suspensions of each microorganism, the methods used by Nascente et al. (2017) were used. Briefly, isolates of cultures of multifunctional microorganisms grown on a solid medium (nutrient agar) were used, and the suspensions were prepared in liquid medium 523 (nutrient broth) (Kado and Heskett, 1970) in a shaking incubator for 24 h at 28°C.

Seed sterilization and microbilization

The seeds were disinfected by soaking in 7.5% sodium hypochlorite for 7 min, and drained after 3 min, afterwards the seeds were soaked in 70% alcohol for 30 min, drained, then rinsed with distilled water three times. The upland rice seeds were dried by placing them in a paper towel contained in a tray in a drying room of temperature at 29°C before microbilization. Microbilization was done with the immersion of the upland rice seeds in the rhizobacterial suspension, and for the control treatment, seeds were immersed in distilled water, for 24 h at a temperature of 25°C, under constant agitation, following the proposed methodology of Filippi et al. (2011).

Planting and germination of upland rice

The seeds were placed into the test tube after drying with the tip of a spatula. Each tube of experimental unit composed of two upland rice seeds cultivar (BRS A501 CL), placed to germinate in a 50 mL volume test tube containing 15 mL of agar-water medium (0.8 m/v). The tubes were placed in a germination room at 28°C with a 12-h photoperiod and removed 10 days after seeding.

Determination of growth parameters

The seedlings were removed from the test tubes, photographed with a camera and image processing was performed. The images obtained were analyzed using WinRHIZO 2012b software. With the software, these parameters were determined: Total Root Length (Comp R, cm), Root Diameter (Diam R, mm), and Root Volume (Vol R, cm³). After recording the parameters, the dry biomass of the rice seedlings was determined after dehydration in a forced air chamber at 65°C until a constant mass was gotten by checking the values on a precision scale.

Statistical analysis

The data were subjected to analysis of variance and, when significance was detected, the means were compared using the Scott Knott test ($p < 0.05$). The SAS statistical package was used to process these data. Additionally, a multivariate principal component analysis (PCA) was performed to describe the correlation between response variables (shoot, root and total biomass, yield components and grain yield) with isolated and mixed microorganisms.

The main components (MCs) were loaded with response variables when the correlation test produced $r > 0.50$. The first three MCs responsible for >62% of the data variation were maintained. Biplots (two-dimensional graph) using these three MCs that correlate isolated and mixed microorganisms and response variables were built with the "FactoExtra" package on the R platform.

RESULTS AND DISCUSSION

The root length (ranged from 6.76 to 26.77 cm), volume (ranged from 0.095 to 0.234 cm³), surface area (ranged from 4.30 to 28.54 m²) and diameter (ranged 0.28 from to 0.60 mm) of upland rice seedlings were affected by microorganisms and differed from the control (no microorganisms) (Figures 1 to 4). The highest values for the root length (Leng R, cm) of the upland rice seedlings were obtained with the treatments: *B. toyonensis* (BRM 32110), *A. brasilense* (AbV5), *S. marcescens* – (BRM 32114) + *B. toyonensis* (BRM 32110), *S. marcescens* (BRM 32114) + *A. brasilense* (AbV5), *B. toyonensis* (BRM 32110) + *A. brasilense* (AbV5), *Bacillus* spp. (BRM 63574) + *B. toyonensis* (BRM 32110), *P. australis* (BRM 62389) + *S. marcescens* (BRM 32114) which were 18.58, 20.74, 26.77, 23.07, 18.12, 16.95, and 20.82 cm, respectively and significantly much higher than that of the control treatment (6.76 cm) (Figure 1). This could be attributed to increased physiological processes of the treated plants relative to untreated ones. According to Nascente et al. (2017), when comparing treated to untreated plants, the treated rice plants had a more effective photosynthesis, stomata permeability, and transpiration process for exchanging gases which could contribute to the growth of rice plants. Furthermore, the combination of *S. marcescens* and *B. toyonensis* led to the greatest increase (296%) in root length relative to the control. This could be attributed to the fact that *S. marcescens* and *B. toyonensis* were able to fix nitrogen, solubilize phosphate and also produce indoleacetic acid (IAA) which aided in the elongation of the root relative the control. It was reported that *S. marcescens* (BRM 32114) was observed to be the most effective microbe in promoting increases in rice shoot biomass (Nascente et al., 2017).

The inoculation of upland rice seedling was observed with M02, M04, M05, M06, M09, M11, M16, M19, M22, M23, M24, M25, and M27 which led to a significantly greater increase in root volume (Vol R), with 0.182, 0.177, 0.203, 0.168, 0.192, 0.170, 0.226, 0.217, 0.227, 0.234, 0.212, 0.190, and 0.204 cm³, respectively, higher than those of the control treatment (Figure 2). Additionally, the mixture of *P. australis* and *Bacillus* spp. greatly increased root volume (47%) in comparison to the control. Fernandes et al. (2020) reported that the use of multifunctional microorganisms significantly affects the total biomass production of the plants, as well as the accumulation of nutrients in the shoots and roots, and in the yield components, resulting in significant increases in

the grain yield of the crop. All sole microorganisms except *S. marcescens*, *P. australis* and *Bacillus* spp. had a significantly higher effect on root volume as compared to the control. However, all combination of *P. australis* with other microorganism except *B. toyonensis* and *T. koningiopsis* significantly increased the root volume of upland rice in comparison to other combinations.

The inoculation of upland with sole and combined microorganism significantly affected the root area (Figure 3). Inoculation of upland rice seedling with M02 (20.85 cm²), M05 (24.27 cm²), M06 (21.31 cm²), M08 (26.45 cm²), M09 (22.97 cm²), M11 (28.54 cm²), M16 (25.58 cm²), M22 (18.32 cm²), M23 (18.93 cm²), M24 (20.53 cm²), M25 (18.00 cm²) and M27 (24.74 cm²) led to a significantly greater total root surface area relative to the control (9.21 cm²) and other treatments. The mixture of *B. toyonensis* and *A. brasilense* greatly increased root surface area by 209% in comparison with the control. Banayo et al. (2012) reported that *A. brasilense* positively influenced the increase in the production of biomass and grain yield of rice crop by producing growth hormones and stimulating greater root development and greater absorption of nutrients, with direct effects on the development of the plant. Among single microorganism treatments, *B. toyonensis*, *A. brasilense*, and *Azospirillum* spp. were superior in terms of effect on root area.

The inoculation of multifunctional microorganism significantly affected the root diameter (Figure 4). This is supported by the findings of Wijayanto et al. (2021) who reported that the use of PGPR was able to increase root length, dry weight of roots and stems, root development, and N content in roots of rice plants. Inoculation of upland rice seedlings with M4 (0.057 mm), M11 (0.048 mm), M16 (0.060 mm), M19 (0.055 mm), M22 (0.053 mm), and M23 (0.056 mm) significantly increased the root diameter relative to the control (0.044 mm) and other treatments. Furthermore, the highest percentage increase in root diameter of upland rice was observed with the inoculation with M16: *A. brasilense* and *Bacillus* spp. The combination of *A. brasilense* and *Bacillus* spp. led to the highest increase in root diameter (36%) of upland rice inoculated in comparison to the control. All treatments except M4, M11, M16, M19, and M23 were similar to the control. Among sole microorganism, only the inoculation of upland rice seedling with *T. koningiopsis* led to significant increases in root diameter. Species of *Trichoderma* have been reported to promote increases of up to 300% in plant growth (Brotman et al., 2010).

With principal component analysis, it can be seen that the variability of treatments with isolated and combined microorganisms with respect to total root length, root diameter, total root surface area, root volume, and root dry mass of rice seedlings treated with multifunctional microorganisms were best described by two principal components (PCs), accounting for 98.1% of the variation in the data, that is, PC1 (62.1%) added to PC2 (36%) (Figure 5). The factor map (biplot) shows groups of

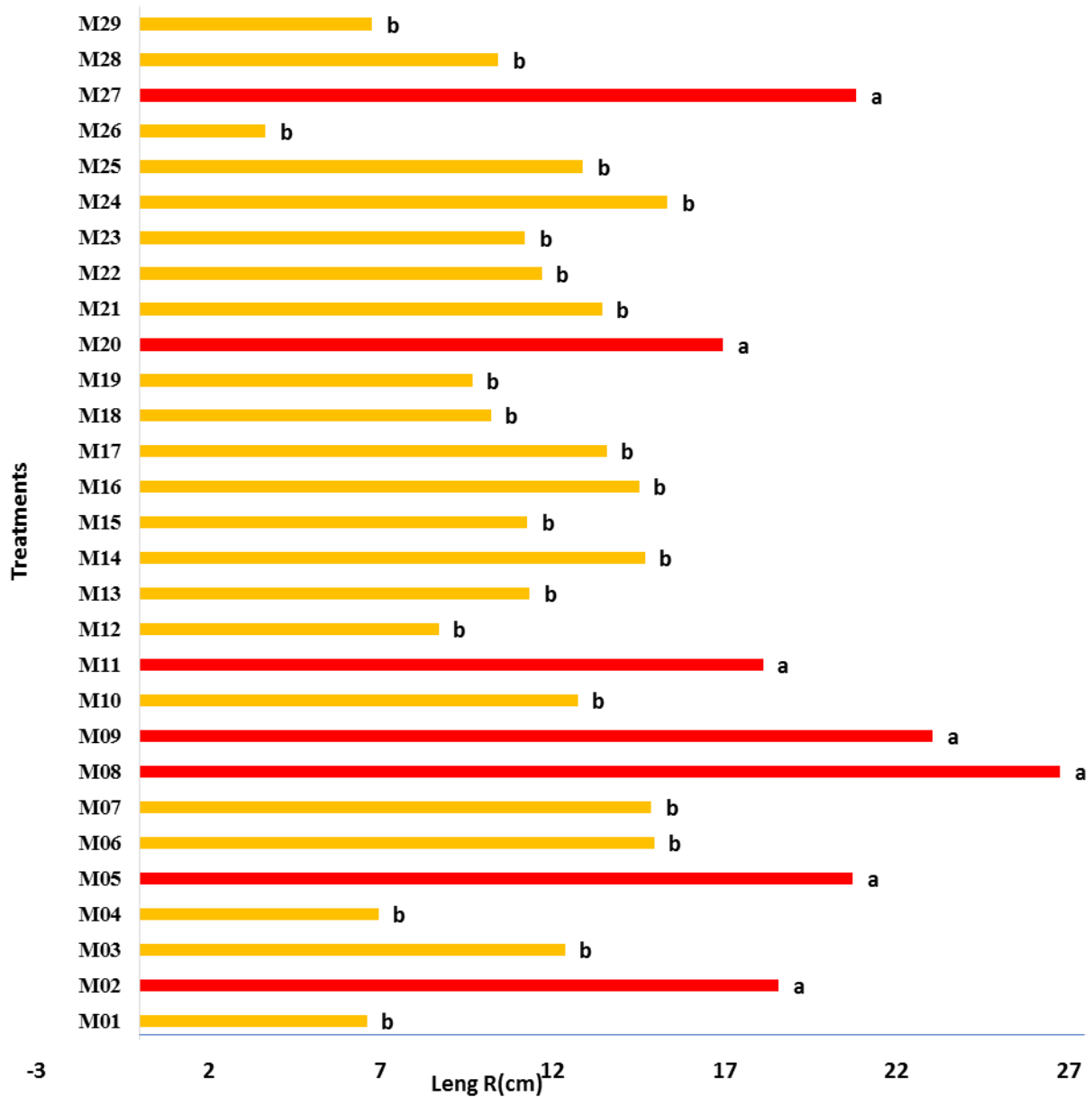


Figure 1. Effect of growth promoting microorganism on total root length of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02- BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*), M05-AbV5 (*Azospirillum brasilense*), M06 -BRM 63574 (*Azospirillum sp.*), M07-BRM 63573 (*Bacillus spp.*), M08-BRM 32114 + BRM 32110 , M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.

Source: Authors

variables (arrows) denoting positive and negative correlations with each principal component (PC), with the length of the arrow indicating the magnitude of each response for each PC (Figure 5B). For example, PC1

was negatively correlated to all variables analyzed. On the other hand, PC2 was positively correlated with root length and root surface area, and negatively correlated with root diameter and root volume.

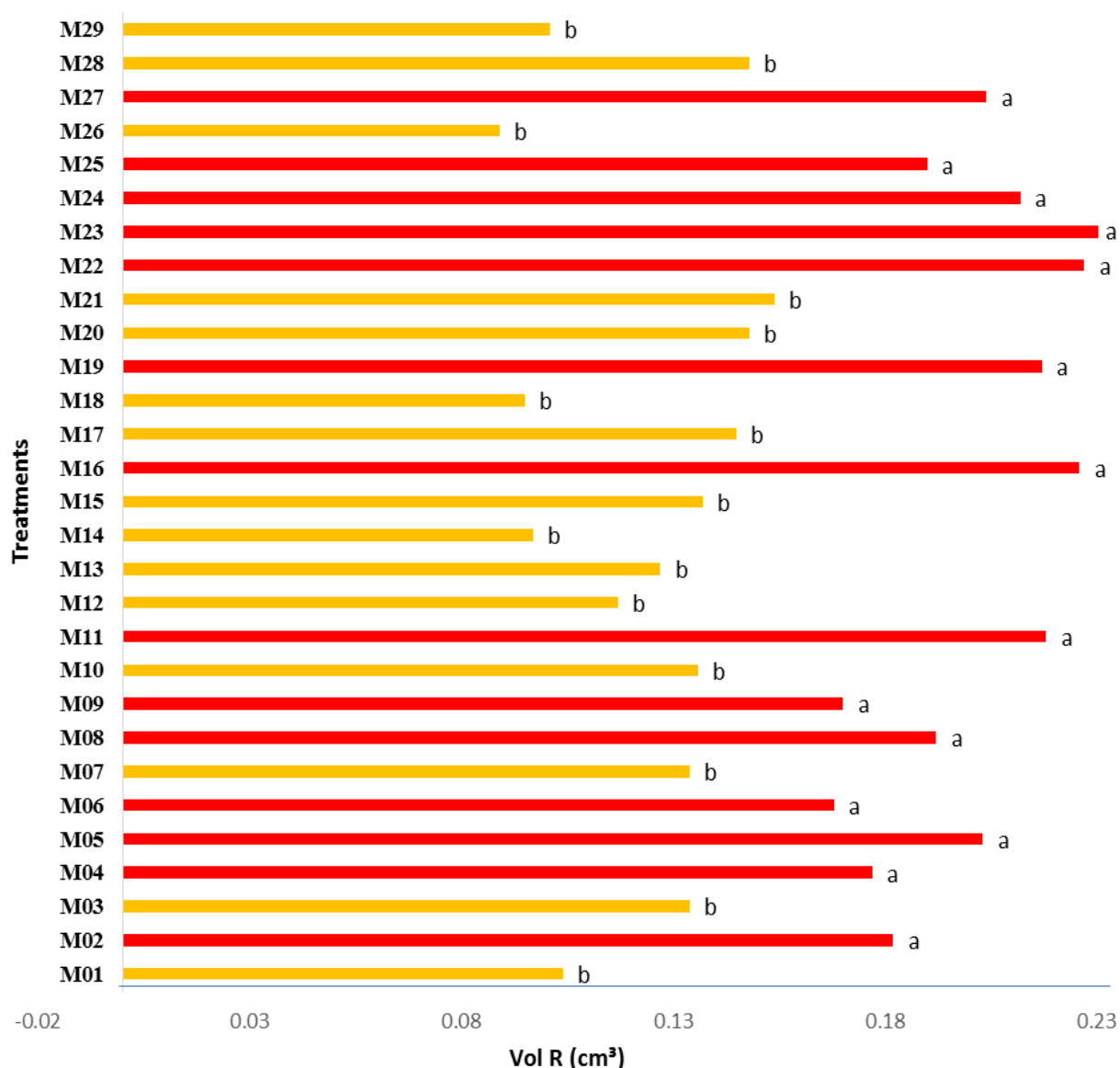


Figure 2. Effect of growth promoting microorganism on root volume of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03-BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*), M05-AbV5 (*Azospirillum brasilense*), M06 -BRM 63574 (*Azospirillum* spp.), M07-BRM 63573 (*Bacillus* spp.), M08-BRM 32114 + BRM 32110, M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

Based on the representational quality of the treatments with microorganisms alone and in combination for the analyzed variables, the treatments M08 and M09, highlighted in purple (Figure 5A), obtained the highest positive correlation for total root length (Figure 5B). The

variable total surface area correlated positively to treatments M02, M05, M06 and M27, highlighted in red. The treatments M11, M24 and M25, in blue, correlated positively with the root volume variable. Regarding the remaining root diameter variable, a positive correlation

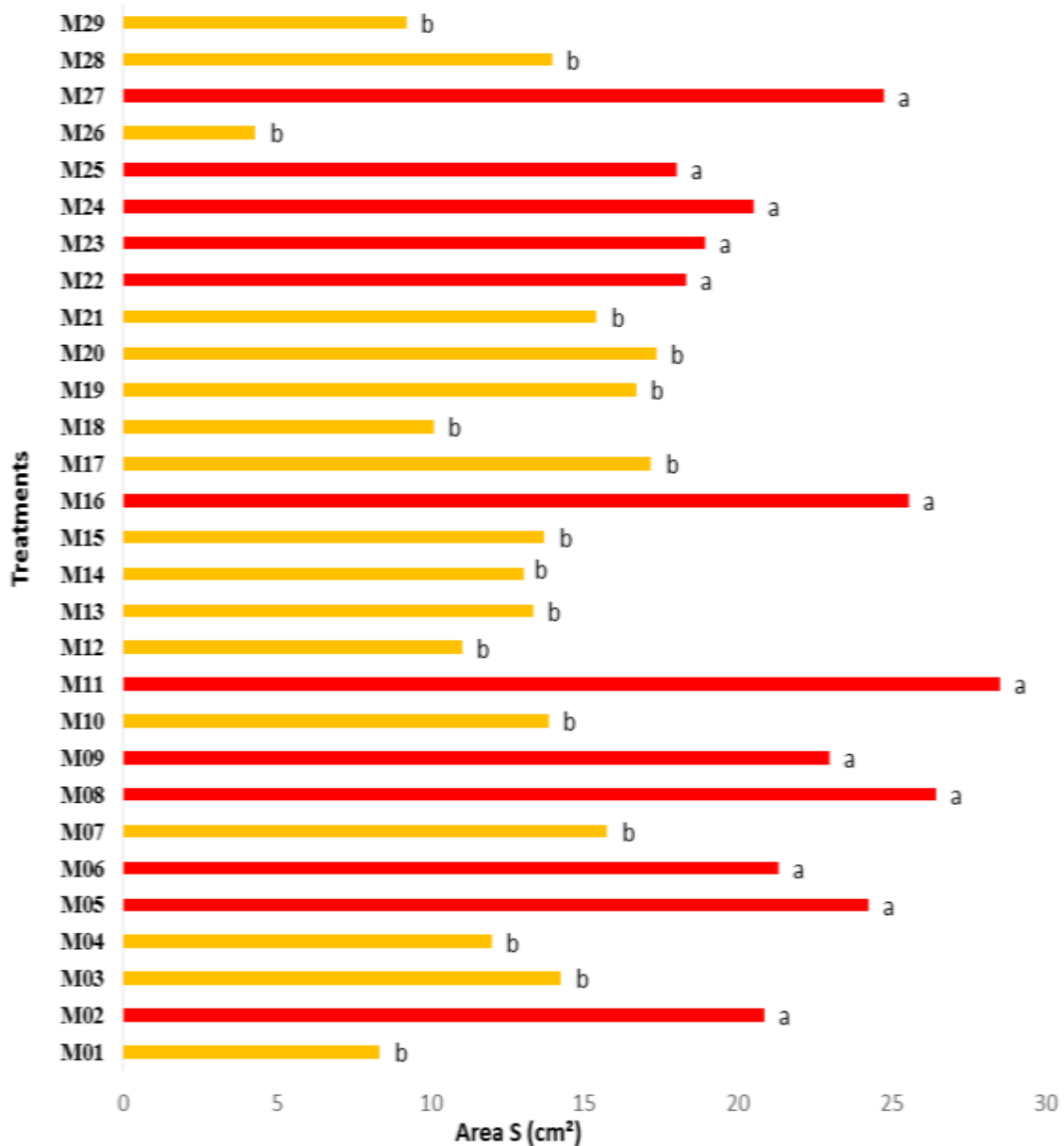


Figure 3. Effect of growth promoting microorganism on surface area of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*), M05-AbV5 (*Azospirillum brasilense*), M06 -BRM 63574 (*Azospirillum* spp.), M07-BRM 63573 (*Bacillus* spp.), M08-BRM 32114 + BRM 32110 , M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.

Source: Authors

was noted with treatments M16, M19, M22 and M23, highlighted in green color. The remaining treatments did not correlate positively with any of the variables analyzed.

The results of the present study show that microbiolization of rice seed with multifunctional microorganisms is a promising approach to improve rice root development.

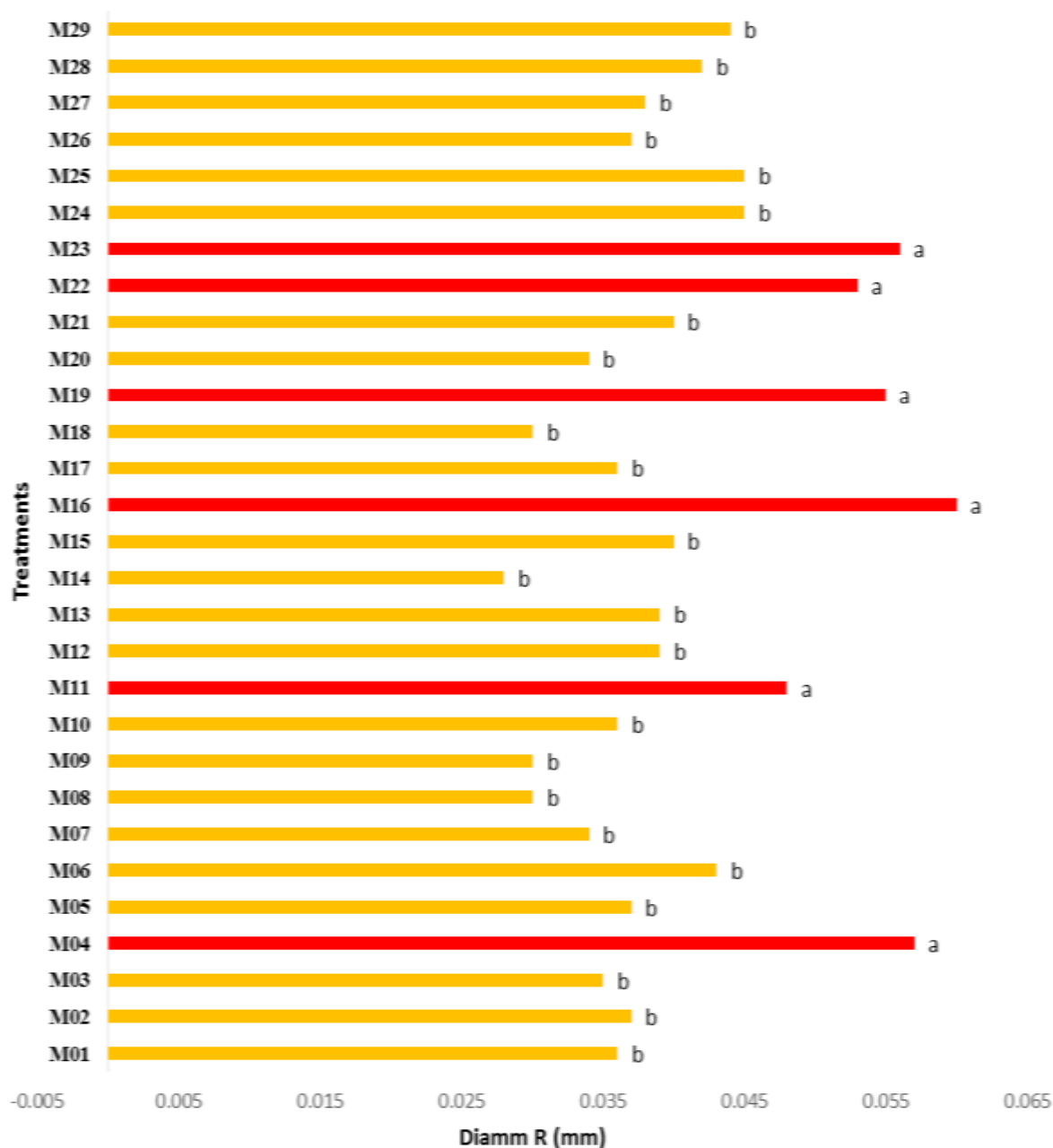


Figure 4. Effect of growth promoting microorganism on root diameter of upland rice seedlings cultivar A 501 CL at ten days after sowing. * Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. ** Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*), M05- AbV5 (*Azospirillum brasilense*), M06 -BRM 63574 (*Azospirillum* spp.), M07-BRM 63573 (*Bacillus* spp.), M08-BRM 32114 + BRM 32110, M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

We could see that these microorganisms enhanced root length, root surface area, root diameter and volume of

upland rice. As a result of greater root development, it is possible that absorption and accumulation of nutrients

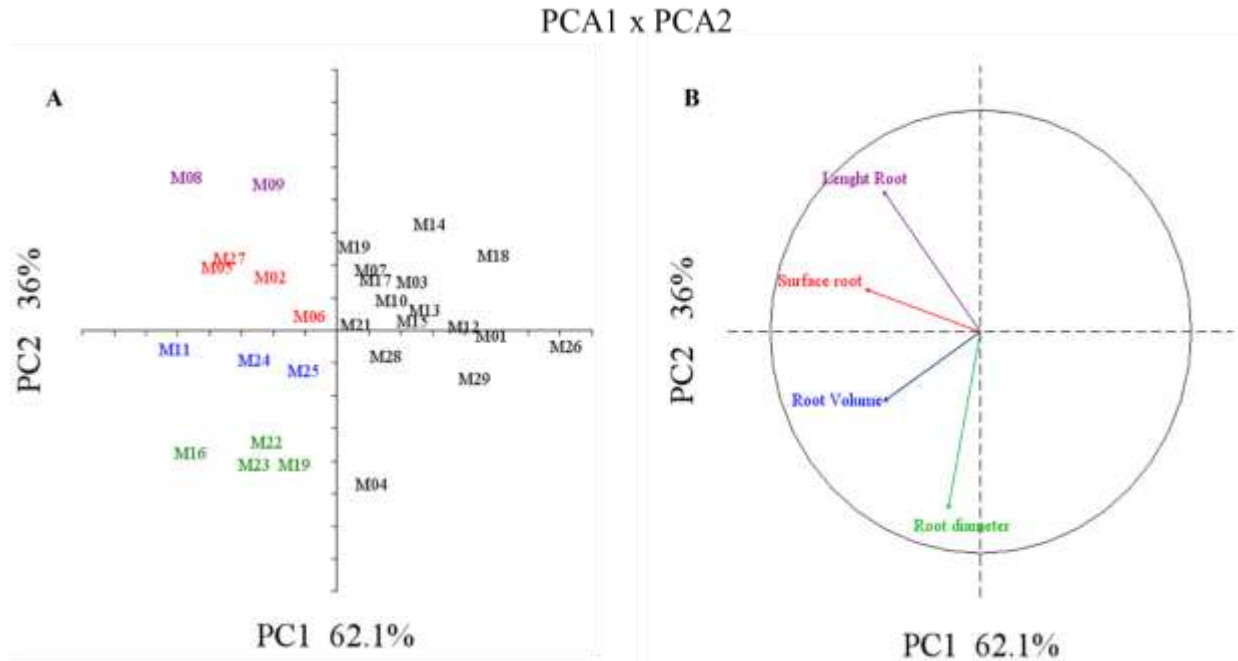


Figure 5. Principal component analysis (PCA) PCA1 X PCA2, explaining the correlations between the evaluated variables and seven treatments with isolates of rhizobacteria and the control (without the microorganism). (A) Biplot graph for treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*), M05-AbV5 (*Azospirillum brasilense*), M06 -BRM 63574 (*Azospirillum* sp.), M07-BRM 63573 (*Bacillus* sp.), M08-BRM 32114 + BRM 32110, M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control. (B) Graph with the variables correlation circle.

Source: Authors

occur because rice plants are able to explore a greater volume of the soil. This will therefore provide a decrease for application of synthetic fertilizer for rice development.

Conclusion

Inoculation of upland rice with sole and combined microorganism on upland rice increased the yield components and grain yield of the crop. The combination of *S. marcescens* and *B. toyonensis* led to the greatest root length while combination of *B. toyonensis* and *A. brasilense* greatly increased root surface area. The highest increase in root diameter was recorded for upland rice inoculated with mixture of *A. brasilense* and *Bacillus* spp. The mixture of *P. australis* and *Bacillus* spp. led to the highest root volume.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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