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RESEARCH ARTICLE

Biomass, gas exchange, and nutrient contents in upland rice plants affected by application forms of microorganism growth promoters

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Abstract Microorganisms are considered a genetic resource with great potential for achieving sustainable development of agricultural areas. The objective of this research was to determine the effect of microorganism application forms on the production of biomass, gas exchange, and nutrient content in upland rice. The experiment was conducted under greenhouse conditions in a completely randomized design in a factorial 7 × 3 + 1, with four replications. The treatments consisted of combining seven microorganisms with three application forms (microbiolized seed; microbiolized seed + soil drenched with a microorganism suspension at 7 and 15 days after sowing (DAS); and microbiolized seed + plant sprayed with a microorganism suspension at 7 and 15 DAS) and a control (water). Treatments with *Serratia* sp. (BRM32114), *Bacillus* sp. (BRM32110 and BRM32109), and *Trichoderma asperellum* pool provided, on average, the highest photosynthetic rate values and dry matter biomass of rice shoots. Plants treated with *Burkolderia* sp. (BRM32113), *Serratia* sp. (BRM32114), and *Pseudomonas* sp. (BRM32111 and BRM32112) led to the greatest nutrient uptake by rice shoots. *Serratia* sp. (BRM 32114) was the most effective for promoting an increase in the photosynthetic rate, and for the greatest accumulation of nutrients and dry matter at 84 DAS, in rice shoots, which differed from the control treatment. The use of microorganisms can bring numerous benefits of rice, such as improving physiological characteristics, nutrient uptake, biomass production, and grain yield.

Keywords *Oryza sativa* · Bioagent · Bio-inducing · Biomass · Growth promoter · Sustainable development

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Introduction

In Brazil, rice can be grown in two types of ecosystems: the lowland and upland conditions with supplemental irrigation or rainwater only (Silva et al. 2010). In upland ecosystem, rice is usually planted in rotation with soybeans, in the renewal of degraded pastures, or as the first crop in opening up new areas for agriculture (Santos et al. 2006). The production of upland rice is growing in importance due to the reduction of irrigation water availability for irrigated lowland rice crops (Nascente et al. 2013).

Among the alternatives for cultivating upland rice, there is the no-tillage system (NTS), which is characterized by maintaining straw on the soil surface in order to provide reduced soil temperature as well as increased levels of soil organic matter and biological activity (D'andréa et al. 2004; Nascente et al. 2013, 2016). However, rice in the NTS has a slow initial growth rate and is more subject to competition imposed by weeds (Nascente et al. 2016). Thus, the start-up and the initial seedling development are important characteristics and need to improve for establishing rice crops in the NTS.

The increases in soil organic matter content in the NTS are one of the main sources of nutrients and energy for an extraordinarily diverse community of soil microorganisms (Ferreira and Martin-Didonet 2012), including bacteria and fungi (Baldani et al. 2000). Rhizodeposition refers to the portion of the soil where the total carbon from the roots are transferred into soil, comprising exudates (smaller molecules) and

secretions (larger molecules such as enzymes), lysates of dead cells, and mucilages. This rich deposition has a high microbial activity, given the abundance of microorganisms present in the soil surface layer (Spaepen et al. 2009). Constituents of the microflora, the plant growth-promoting rhizobacterias (PGPR), have important molecules within their cell constitution, such as flagellin (protein constituent of flagella) and lipopolysaccharides (LPS, cell membrane constituents) (Kaschuk et al. 2010). These components are recognized by the plant as metabolism activators. Thus, the benefits of the interaction between PGPR and plants can be direct, such as growth promotion, since some bacterial groups produce phytohormones, such as indole acetic acid (IAA), cytokinins, and gibberellins, solubilize phosphorus, and produce siderophores (Spaepen et al. 2009; Ahemad and Kibret 2014). Among the indirect benefits, there is the protection against pathogens, such as the induction of resistance and direct antagonism (Filippi et al. 2011).

These PGPRs are basic components of food webs and play crucial and unique roles in the agricultural production system (Anees et al. 2010). Several bacterial genera are currently being studied as biostimulants and biofertilizers, such as *Azospirillum* strain AbV5 (Isawa et al. 2010), *Gluconacetobacter* (Muthukumarasamy et al. 2005), *Herbaspirillum*, *Burkholderia* (Baldani et al. 2000), *Pseudomonas* (Yao et al. 2010), *Bacillus*, *Serratia*, *Paenibacillum*, *Enterobacter*, and *Klebsiella* (Spaepen et al. 2009). Thus, the association between PGPR and many crops, such as sugarcane, *Saccharum officinarum* (Lopes 2013); corn, *Zea mays* (Dartora et al. 2013); common beans, *Phaseolus vulgaris* (Martins 2013); eucalyptus, *Eucalyptus grandis* (Moreira and Araújo 2013); and rice, *Oryza sativa* (Souza Júnior et al. 2010), resulted in plant growth promotion without nitrogen fertilizer supplementation, in addition to reducing the occurrence of diseases.

Research focusing on PGPRs, which naturally occurs as potential biostimulants for rice plants, has shown promising results from an agricultural point of view (Isawa et al. 2010). During studies conducted at Embrapa Arroz e Feijão, promising rhizobacteria isolates (BRM32113, BRM32111, BRM32114, BRM32112, BRM32110, and BRM32109) were selected, because they have shown promise mainly due to their increased biomass production and disease suppression (Filippi et al. 2011; Silva et al. 2012; França et al. 2015).

Additionally, during studies conducted at the Federal Rural University of Amazon, four *Trichoderma asperellum* isolates were selected and tested as growth promoters and biocontrol agents, both in the greenhouse and in the field (Silva et al. 2012; Ferrari et al. 2013; França et al. 2015). However, there is still little information about what effects these bioagents (rhizobacterias isolates and *T. asperellum*) have on the gas exchange and nutrition of rice plants in upland regions. Therefore, this paper brings innovation due to making comparison of bioagents' beneficial effects in promoting the growth of upland rice, which is done by assessing the health of the plant (gas exchange and nutritional status).

The hypothesis of this study is that the previously selected bioagents, when applied, either via seed or soil/foliar spray, significantly affect the development of rice plants, which is reflected in gas exchange, biomass production, and nutrient concentration. The objective was to determine the effect of different methods of applying microorganisms, previously identified as growth inducers, on the production of biomass dry matter, gas exchange, and nutrient content of upland rice plants.

Materials and methods

Environment characterization

The experiment was conducted in a greenhouse at the Embrapa Arroz e Feijão research center, Santo Antônio de Goiás, GO, Brazil. We used soil from the arable layer (0–0.20 m) of a clay loam (kaolinitic, thermic Typic Haplorthox) acidic soil from a *Brachiaria brizantha* pasture that had been in use for 20 years, with 377, 260, and 363 g kg^{-1} of sand, silt, and clay, respectively. The chemical characteristics of the soil were determined according to the methods described by Claessen (1997). The results were pH (H_2O) = 5.8; hydrolytic acidity = 46.4 $mmol_c$ kg^{-1}; Ca^{2+} = 11.61 $mmol_c$ kg^{-1}; Mg^{2+} = 1.38 $mmol_c$ kg^{-1}; Al^{3+} = 1.0 $mmol_c$ kg^{-1}; $H^+ + Al^{3+}$ = 25.0 $mmol_c$ kg^{-1}; P = 0.2 mg kg^{-1}; K^+ = 45 mg kg^{-1}; Cu^{2+} = 2.1 mg kg^{-1}; Zn^{2+} = 0.2 mg kg^{-1}; Fe^{3+} = 14.3 mg kg^{-1}; Mn^{2+} = 6.0 mg kg^{-1}; total exchangeable bases = 14.14 $mmol_c$ kg^{-1}, total N = 0.9 g kg^{-1}; and soil organic matter = 17.93 g kg^{-1}.

Three weeks before the rice planting, pots were filled with 7 kg of soil fertilized with 70 mg dm^{-3} of N (urea), 400 mg dm^{-3} P_2O_5 (simple superphosphate), and 200 mg dm^{-3} of K_2O (potassium chloride). Soil moisture, throughout the experiment, was monitored daily by weighing the pots. The evapotranspirated moisture was replaced when it reached 85%, increasing it to 100% of the field capacity.

Experimental design and treatments

The experimental design was completely randomized in a factorial 7 × 3 + 1, with four replications. The treatments consisted of combining seven microorganisms, six rhizobacteria isolates and a *T. asperellum* pool with three application form. The rhizobacterial isolates (BRM 32113, 32111, 32112, 32114, 32110, and 32109) are described in Table 1, and they are currently stored and preserved in the Multifunction Microorganisms and Fungi collection from the Embrapa Arroz e Feijão. The *T. asperellum* pool is composed of four isolates T-06, T-09, T-12, and T-52, which were isolated from rhizospheric soils of reforested and native forest

Table 1 Collection code, origin, some biochemical characteristics, and taxonomic classification of the six rhizobacteria isolates utilized for seed and plant treatments

Code[a]	Origin[b]	Color[c]	Biochemical[c]					Taxonomic[c]
			AIA[d]	Cellulase[e]	Phosf[f]	Sider.[g]	Biofilm[h]	
BRM32111	PA/Brazil	Yellow		+	+	+	+	*Pseudômonas* sp.
BRM32113	PA/Brazil	Pink	+	+		+	+	*Burkholderia* sp.
BRM32114	PA/Brazil	Pink	+	+	+	+	+	*Serratia* sp.
BRM32112	GO/Brazil	Yellow		+	+	+	+	*Pseudomonas* sp.
BRM32109	GO/Brazil	White		+	+		+	*Bacillus* sp.
BRM32110	PA/Brazil	White		+	+	+	+	*Bacillus* sp.

[a] Number code of rhizobactera isolates in the Microorganisms and Fungi Multifunction Embrapa Rice and Beans collection

[b] Geographical origin of each isolate

[c] Colony color, biochemical characterization, and taxonomic classification of each isolate, described by Martins (2015)

[d] Acid endol acetic producer

[e] Cellulase producer

[f] Phosfatase producer

[g] Sideropher producer. The methodology is described in Martins (2015)

[h] Bacteria that produce biofilm

areas in the Amazon, taxonomically identified by Ferrari et al. (2013), and currently stored and preserved in the Fungal Culture Collection of the Plant Protection Laboratory at the Federal University Rural of Amazon.

There were three application forms: (1) seed—microbiolized seed; (2) seed-soil—microbiolized seed + soil drenched with microorganism suspension at 7 and 15 days after sowing (DAS); and (3) seed-plant—microbiolized seed + plant sprayed with microorganism suspension at 7 and 15 DAS. The control treatment consisted of no microbiolized seed and soil drenched and plant sprayed with only water without any microorganism.

Seed microbiolization

The bacterial suspensions were prepared with water, from cultures that had been growing for a 24-h period on solid medium 523 (Kado and Heskett 1970), at 28 °C, and the concentration was set in a spectrophotometer to A540 = 0.5 (10^8 UFC). The rice seeds were immersed in each suspension, and the control seeds were immersed in water for a period of 24 h under constant agitation at 25 °C.

Each isolate of *T. asperellum* pool was grown in a Petri dish containing potato dextrose agar (PDA) for 5 days and bioformulated as described by Silva et al. (2012). The seed treatment was performed at concentrations of 10 g of powdered (Silva et al. 2012) *T. asperellum* per 1 kg of seed (Filippi et al. 2011; Silva et al. 2012). The concentration of the biological suspension was 10^8 conidia ml^{-1}.

- *Soil drench*: 100 ml of suspension of each treatment, all the bacterial isolates (10^8 CFU), the *T. asperellum* pool (10^8 conidia. ml^{-1}), and water drenched the trial soil at 7 and 15 DAS.
- *Plant spray pulverization*: 30 ml of suspension of each treatment, all the bacterial isolates (10^8 CFU), the *T. asperellum* pool (10^8 conidia ml^{-1}), and water were sprayed on the plants, using a manual backpack sprayer, at a constant pressure provided by a CO_2 pressure source and a conical nozzle type (TX-VS2), at 7 and 15 DAS.

Rice plant management

Ten rice seeds were sown per pot (mutant genotype 07SEQCL441 CL was derived from a Primavera variety and was resistant to Imazapyr + Imazapic herbicide) on September 10, 2015. Plant emerged on September 15, 2015. Ten days after germination, we thinned the shoots and kept three plants per pot. At the beginning of the rice tillering stage, a topdressing fertilization (1 g of ammonium sulfate per pot) was performed. Weed control was performed manually, and there was no incidence of any insects or diseases.

Assessments

Gas exchange

The gas exchange and sheet width (cm) were sampled from the rice plants. Therefore, we measured photosynthetic rate, A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$); transpiration rate, E (mmol $H_2O\ m^{-2}\ s^{-1}$); stomata conductance, gs (mol $H_2O\ m^{-2}\ s^{-1}$); internal CO_2 concentration, Ci (vpm); and leaf temperature, Tleaf (°C), determined by a portable gas meter in the infrared region IRGA (LCpro, ADC BioScientific), from 08:00 to 10:00 am, at 33 and 66 DAS.

Samples were taken in the middle third of the first fully expanded leaf (top to base) during the two evaluation periods. The equipment was set to use concentrations of 370–400 mol mol^{-1} CO_2 in the air, which is the reference condition used in the IRGA photosynthesis chamber. The photon flux density photosynthetic active (PPFD) used was 1200 μmol [quanta] m^{-2} s^{-1}. The minimum equilibration time set for performing the reading was 2 min.

Biomass production

The shoots of rice plants were collected for biomass assessment 84 days after seed emergence, when 50% of plants were in full flower stage. Thus, in each treatment, the plants were dried at 65 °C, until constant weight, and weighed to determine the dry matter of the biomass.

Concentration of nutrients in rice plants

Following the collected plants' drying and weighing, aliquots were taken which were grounded and an analysis of the nutrient content (N, P, K, Ca, Mg, Fe, Zn, Cu, and Mn) was performed in the rice shoots in accordance with recommendations set out by Malavolta et al. (1997).

Statistical analysis

All data were analyzed using analysis of variance, and *F* probability test was performed. The microorganism species and their application forms were considered fixed effects. Blocks and all block interactions were considered random effects. A comparison of means was performed with Tukey's test ($p \leq 0.05$). Dunnett's test was performed at a significance of $p \leq 0.05$ to compare the treatment without microorganism (control) with each treatment with microorganism application. We used the SAS statistical package (SAS 1999).

Results

Physiological characterization of the gas exchange process

The gas exchanges were more pronounced when rice plants were treated with growth-promoting microorganisms (Table 2). The photosynthetic rate (A) was significantly higher than that in the control, especially for isolates BRM32110 (16.5%), *T. asperellum* pool (15.2%), BRM32109 (13.5%), BRM32112 (7.9%), and BRM32114 (6.4%). The transpiration rate (E) values of rice plants treated with the isolate BRM32113 were similar to the control treatment. Plants treated with the isolates BRM32110, *T. asperellum* pool, BRM32109, and BRM32111 presented the highest E. Regarding stomata conductance (gs), plants treated with the isolate BRM32110 presented the highest values and differed statistically from plants treated with isolates BRM32113 and BRM32112, as well as the control treatment (Table 2). There was no statistical difference among plants treated with isolates and the control for internal CO_2 concentration (Ci) and leaf temperature (Tleaf).

The three different microorganism application forms did not affect leaf width, photosynthesis, stomata conductance, and leaf temperature (Table 2). Moreover, the application form affected transpiration, which was higher in the microbiolized seed and sprayed plant treatments, both statistically different from only microbiolized seed treatment. On the other hand, internal CO_2 concentration (Ci) was also affected by application form; the highest values were observed in plants from microbiolized seeds only.

Shoot dry biomass

Concerning shoot dry biomass, there were differences observed among treatments (Table 3). The isolate BRM32114 provided the largest increase (21.53 g), which differed from the control (18.54 g). The isolate BRM32111 provided a reduction in rice biomass (11.24 g) and was also different from the control treatment. The remaining isolates did not provide significant increases in biomass production, based on the control treatment.

The application form of the three isolates (BRM32111, BRM32114, and BRM32112) did not affect the dry matter accumulation of rice plant shoots (Table 4). On the other hand, dry matter was higher in rice plants when the isolates BRM32113 and *T. asperellum* pool were applied by seed-soil or by seed-plant. The isolate BRM32110 applied by seed-soil provided a lower shoot dry matter than that applied by the seed. Similar results were observed from the isolate BRM32109, when applied by seed-plant; it provided lower biomass values, statistically different from the seed only treatment. In all three application forms, the isolate BRM32114 provided the highest dry biomass values, while the isolate BRM32111 provided the lowest biomass values of rice shoots.

Nutrient content in rice plants

Regarding nutrient levels in treated rice plants, microorganisms and application form had no interaction for K, Mg, and Fe absorption (Table 5). Thus, all three application forms and isolates did not affect K content in rice plants. On the other hand, rice plants treated with the isolate BRM32112 increased Mg absorption, which was different from plants treated with isolates BRM32113 and BRM32111. Regarding Fe absorption, rice shoots treated with the isolate BRM32111 presented the highest levels of this micronutrient and was different from isolates BRM32113, BRM32114, BRM32110, and BRM32109. Mg contents were higher in plants when microorganisms were applied by seed and by soil than by seed only

Table 2 Microorganism application method affects leaf width (LW), photosynthesis (A), transpiration (E), stomata conductance (gs), internal CO_2 concentration (Ci), and leaf temperature (Tleaf) of rice plants in two evaluation times (33 and 66 days after sowing, DAS)

Factors	LW (cm)	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	Ci (vpm)	Tleaf (°C)
Microorganism						
BRM32113	1.22^{ab}	15.97^{c}	6.08*^{c}	0.34^{b}	279	31.81^{ab}
BRM32111	1.18*^{b}	16.29^{bc}	6.66^{abc}	0.36^{ab}	280	32.33^{ab}
BRM32114	1.28^{a}	16.98^{abc}	6.30^{bc}	0.36^{ab}	279	31.73^{ab}
BRM32112	1.26^{a}	17.23^{abc}	6.35^{bc}	0.33^{b}	275	31.47^{b}
BRM32110	1.24^{ab}	18.59^{a}	7.19^{a}	0.39*^{a}	270	32.30^{ab}
BRM32109	1.29^{a}	18.12^{ab}	6.84^{ab}	0.36^{ab}	269	32.44^{a}
T. asperellum pool	1.28^{a}	18.38^{a}	6.53^{abc}	0.36^{ab}	274	32.17^{ab}
Control	1.35	15.96	6.06	0.32	280	31.56
Application form						
Seed	1.22	17.17	6.34^{b}	0.35	279^{a}	31.89
Seed-soil	1.26	17.48	6.53^{ab}	0.36	275^{ab}	31.82
Seed-plant	1.27	17.44	6.82^{a}	0.36	271^{c}	32.40
Evaluation time						
33 DAS	0.90^{b}	17.80^{a}	8.49^{a}	0.29^{b}	257^{b}	33.74^{a}
66 DAS	1.60^{a}	16.93^{a}	4.64^{b}	0.42^{a}	294^{a}	30.33^{b}
ANOVA (*F* probability)						
Microorganism (M)	0.0889	0.0281	0.0604	0.0966	0.3767	0.2652
Aplication form (F)	0.1757	0.8551	0.1310	0.6945	0.1055	0.1069
Evaluation time (E)	<0.001	0.0804	<0.001	<0.001	<0.001	<0.001
M × F	0.3622	0.5250	0.2188	0.8800	0.3885	0.3065
M × E	0.8057	0.0361	0.1753	0.3426	0.3666	0.9604
F × E	0.8448	0.7618	0.4049	0.6314	0.3951	0.6885
M × F × E	0.4809	0.9139	0.9117	0.7498	0.7820	0.7911
CV (%)	11.73	18.53	19.22	18.05	7.59	4.94

Means followed by the same letter do not differ by Tukey's test at $p \leq 0.05$. Means followed by asterisk differ from the control treatment (no microorganism) by Dunnett's test at $p \leq 0.05$

CV coefficient of variation

application. The highest values for Fe content were obtained when isolates were applied in the seed only or by seed-plant.

Regarding levels of N, P, Ca, Cu, Mn, and Zn nutrients, the statistical analyses revealed a significant interaction between microorganisms and application form (Table 6). Thus, the isolate BRM32111 applied only by seeds presented the highest N contents in rice plants and differed from other isolates. The isolate BRM32109, applied by seed and by soil, allowed the highest N contents, and isolates BRM32111 and BRM32110 applied by seed and by plant provided the statistically highest contents of N. However, for the majority of isolates (BRM32113, BRM32111, BRM32114, BRM32112, and *T. asperellum* pool), it was observed that seed application form provided the best results of N content in the rice shoots.

Higher levels of P were observed in the rice plant shoots when the isolate BRM32111 was applied to the seed. On the other hand, rice plants presented the lowest level of this nutrient when treated by the isolate BRM32109 (Table 6). The seed-soil application of isolates BRM32112, BRM32109, BRM32114, and *T. asperellum* pool provided the highest values of P contents, different from treatment with isolates BRM32113, BRM3211, and BRM32110. The isolate BRM32112 applied by seed-plant application provided the highest values and differed from isolates BRM32113, BRM32114, BRM32110, BRM32109, and *T. asperellum* pool. Isolates BRM32113, BRM32111, BRM32114, BRM32110, and *T. asperellum* pool applied by the seed provided the highest P content in rice plants and isolates BRM32112 and BRM32109 when applied by seed-plant and seed-soil.

T. asperellum pool applied in the seed provided the highest levels of Ca in the rice plant shoots, which differed from the isolates BRM32113 and BRM32112 (Table 6). When the isolate BRM32109 was applied by seed-soil, plants obtained the highest values of Ca, which was different from isolates BRM32113, BRM32111, BRM32114, BRM32112, and *T. asperellum* pool. When the isolate BRM32110 was applied

Table 3 Application form of microorganisms affecting biomass dry matter of rice shoots

Factors	Shoot dry biomass (grams)
Microorganism	
BRM32113	18.02[b]
BRM32111	11.24[c]*
BRM32114	21.53[a]*
BRM32112	18.88[b]
BRM32110	19.37[ab]
BRM32109	19.30[ab]
T. asperellum pool	19.04[ab]
Control	18.54
Application form	
Seed	17.74[a]
Seed-soil	18.50[a]
Seed-plant	18.35[a]
ANOVA (*F* probability)	
Microorganism (M)	<0.001
Application form (F)	0.6389
M × F	0.0005
CV (%)	17.29

Means followed by the same letter do not differ by Tukey's test at $p \leq 0.05$. Means followed by asterisk differ from the control treatment (no microorganism) by Dunnett's test at $p \leq 0.05$

CV coefficient of variation

by seed-plant, higher levels of Ca in rice plant shoots were obtained, differing from isolates BRM32113, BRM32111, BRM32109, and *T. asperellum* pool. The application of BRM32113, BRM32111, BRM32109, and *T. asperellum* pool only by seed was more effective to raise Ca content in the rice plant shoots. In plants treated with isolates BRM32112 and BRM32110, Ca contents were similar for both application forms, by seed-soil and by seed-plant, while for the isolate BRM32114, the best result was obtained by seed-plant application.

For Cu, all isolates provided similar levels in the rice plant shoots when isolates were applied by the seed (Table 6). However, the isolate BRM32111 was the most efficient and different from others when applied by seed-soil. The isolate BRM32112, applied by seed-plant, allowed the best results differing from isolates BRM32114, BRM32109, and *T. asperellum* pool.

Plants treated with the isolate BRM32111 allowed the highest content of Mn in rice plant shoots when the application was by the seed, which differed from isolates BRM32113, BRM32110, and BRM32109 (Table 6). The isolate BRM32111 also provided the highest values and differed from the other bioagents, when applied by seed-soil. For the seed-plant application, plants treated by isolate BRM32113 provided the best results differing from isolates BRM32109 and *T. asperellum* pool. The application of isolates only in seeds resulted in higher Mn content in rice plants, when treated with isolates BRM32113, BRM32114, BRM32112, and *T. asperellum* pool, while, for the isolate BRM32111, it was the seed-soil application, and for isolates BRM32110 and BRM32109, it was the seed-soil or seed-plant application that provided the highest Mn content in the rice plant shoots.

The isolate BRM32110 provided the highest Zn content in the rice plant shoots, when the application was made only by the seed, and was different from the isolate BRM32113 (Table 6). The highest values were obtained with the *T. asperellum* pool, which differed from the microorganisms BRM32113 and BRM32111. In the seed-soil application, the highest levels of Zn were found in the plants treated with the isolate BRM32110, which differed from all other five isolates, when the application was seed-soil or seed-plant. For isolates BRM32113, BRM32111, BRM32110, BRM32109, and

Table 4 Application form of microorganisms affecting biomass production of rice shoots

Factors	Shoot dry biomass (grams)				
Microorganism	Seed	Seed-soil	Seed-plant	CV (%)	*F* value
BRM32113	12.71[cd B]	18.79[a A]	22.55[a A]	13.76	0.0456
BRM32111	10.76[c A]	12.02[b A]	10.94[e A]	16.43	0.4657
BRM32114	22.09[a A]	20.89[a A]	21.60[ab A]	15.47	0.3147
BRM32112	20.22[ab A]	17.78[ab A]	18.63[c A]	11.33	0.8414
BRM32110	20.88[a A]	17.77[ab B]	19.47[bc AB]	8.92	0.0315
BRM32109	21.02[a A]	22.60[a A]	14.28[d B]	11.49	<0.001
T. asperellum pool	16.51[bc B]	19.62[a AB]	20.99[ab A]	12.24	0.0241
CV (%)	14.53	24.43	8.45	–	–
ANOVA (*F* probability)	<0.001	0.0318	<0.001	–	–

Means followed by the same letter, lowercase in column or upper case in line, do not differ by Tukey's test at $p \leq 0.05$

CV coefficient of variation

Table 5 Application form of microorganisms affecting nutrient content in rice shoots

Factors	N g kg^{-1}	P	K	Ca	Mg	Cu mg kg^{-1}	Fe	Mn	Zn
Microorganism									
BRM32113	25*	2.1	21^{a}	4.7*	3.4^{bc}	7.9	123^{c}	1766	42
BRM32111	25*	2.3	23^{a}	4.4*	3.3^{c}	10.3	169^{a}	2190*	41
BRM32114	24	2.1	23^{a}	4.8	3.6^{abc}	7.2	136^{bc}	1666	43
BRM32112	24	2.4	22^{a}	5.1	3.8^{a}	9.0	161^{ab}	1799	44
BRM32110	23	2.1	21^{a}	5.2	3.6^{abc}	8.7	124^{c}	1637	48
BRM32109	25*	2.2	21^{a}	5.2	3.7^{ab}	8.4	133^{bc}	1657	43
T. asperellum pool	24	2.4	22^{a}	5.0	3.6^{ab}	8.4	155^{ab}	1717	45
Control	23	2.1	21	5.6	3.6	8.7	122	1864	43
Application form									
Seed	25	2.3	21^{a}	4.8	3.5^{b}	8.7	151^{a}	1644	42
Seed-soil	23	2.1	22^{a}	4.9	3.7^{a}	8.5	115^{b}	1904	43
Seed-plant	24	2.2	22^{a}	5.0	3.5^{ab}	8.4	162^{a}	1762	46
ANOVA (*F* probability)									
Microorganism (M)	0.0036	0.0077	0.4377	0.0242	0.0623	0.0118	0.0072	<0.001	0.1073
Application form (F)	<0.001	0.2115	0.3248	0.6570	0.1075	0.8648	<0.001	<0.001	0.0222
M × F	<0.001	0.0080	0.7475	0.0002	0.0593	0.0054	0.1368	<0.001	0.0092
CV (%)	5.25	11.54	12.34	12.41	10.73	19.22	14.19	11.12	12.93

Means followed by the same letter do not differ by Tukey's test at $p \leq 0.05$. Means followed by asterisk differ from the control treatment (no microorganism) by Dunnett's test at $p \leq 0.05$

CV coefficient of variation

T. asperellum pool, the best form of application to allow high Zn uptake is microbiolization of rice seeds. In plants treated with the isolate BRM32114, the highest values of Zn in rice shoots were found in the seed-soil application, and for those treated with the isolate BRM32112, the highest were found in the seed-plant application.

While comparing the contents of nutrients in the control, the Ca absorption by rice plants was reduced in the treatments with isolates BRM32113 and BRM32111 (Table 5). On the other hand, the Mn absorption was increased when the rice plants were treated with the bioagent BRM32111. In addition, the highest absorption of N was obtained when the rice plants were treated with isolates BRM32113, BRM32111, and BRM32109.

Discussion

The application forms, on average, showed similar results. Then, the application only in the seeds would be enough to provide effects of the microorganisms on upland rice plants development. It should be easier and cheaper to the farmers once they need only this application during plant development. Results also demonstrated that the treated rice plants had a more efficient gas exchange process, which includes photosynthesis, stomata conductance, and transpiration, when compared with the non-treated plants (control plants). These results corroborate our research hypothesis; the microorganisms utilized positively affected the physiological parameters of the rice plants and could have significant effects on biomass production and crop yields. The ability to photosynthesize, and the ability of the stomata to drive water in a vapor form (from leaf to atmosphere) in a given time (stomata conductance), has been intensively studied since these parameters determine the initial process for the biomass production. *Bacillus* sp. isolate (BRM32110) provided the highest photosynthetic rate and stomata conductance to rice plants and did not differ from *Serratia* sp. (BRM32114), *Bacillus* sp. isolate (BRM32109), and *T. asperellum* pool. This suggests that these microorganisms positively influenced the stomata opening and closing, which directly affected the process of gas exchange and contributed to a greater accumulation of biomass. Concerning the internal CO_2 concentration, there were no differences among the plants, both treated and untreated, indicating that CO_2 concentration was the same as in plants that are correctly irrigated, regardless of the presence of plant growth-promoting microorganisms. Souza et al. (2015) observed increases of up to 176% of the content of total sugars in rice plants after seed-soil application of isolates BRM32113 (*Burkholderia* sp.), BRM32111 (*Pseudomonas* sp.), and *T. asperellum* pool. The physiological effects caused by microorganisms on plant growth include changes in many processes in which range from the dynamics of gas exchange

Table 6 Application form of microorganisms (seed (S), seed-soil (SS), and seed-plant (SP)) affecting the nutrient content in rice shoots

Factor	S	SS	SP	*F* value	S	SS	SP	*F* value
Microorganism	N content (g kg^{-1})				P content (g kg^{-1})			
BRM32113	$26^{b\ A}$	$23^{b\ B}$	$24^{bc\ AB}$	0.0332	$2.2^{ab\ A}$	$2.0^{b\ A}$	$2.0^{c\ A}$	0.5427
BRM32111	$28^{a\ A}$	$20^{c\ C}$	$25^{a\ B}$	<0.001	$2.6^{a\ A}$	$2.0^{b\ C}$	$2.4^{ab\ B}$	<0.001
BRM32114	$25^{bc\ A}$	$23^{b\ A}$	$24^{bc\ A}$	0.6783	$2.2^{ab\ A}$	$2.2^{a\ A}$	$2.0^{c\ B}$	0.0418
BRM32112	$26^{b\ A}$	$23^{b\ B}$	$23^{c\ B}$	<0.001	$2.2^{ab\ B}$	$2.4^{a\ AB}$	$2.6^{a\ A}$	0.0406
BRM32110	$21^{d\ B}$	$22^{bc\ B}$	$25^{a\ A}$	0.0404	$2.1^{ab\ AB}$	$1.9^{b\ B}$	$2.3^{b\ A}$	0.0379
BRM32109	$23^{c\ B}$	$28^{a\ A}$	$24^{bc\ B}$	<0.001	$1.9^{b\ B}$	$2.4^{a\ A}$	$2.1^{bc\ AB}$	0.0221
T. asperellum pool	$25^{bc\ A}$	$24^{b\ A}$	$23^{c\ A}$	0.2867	$2.6^{ab\ A}$	$2.3^{a\ A}$	$2.3^{b\ A}$	0.2974
ANOVA (*F* probability)	<0.001	0.0348	0.0412	–	0.0487	0.0319	<0.001	–
Microorganism	Ca content (g kg^{-1})				Cu content (mg kg^{-1})			
BRM32113	$4.3^{b\ A}$	$4.9^{bc\ A}$	$4.9^{b\ A}$	0.2515	$7.4^{a\ A}$	$6.8^{d\ A}$	$9.6^{ab\ A}$	0.2879
BRM32111	$5.0^{ab\ A}$	$3.6^{d\ B}$	$4.7^{bc\ A}$	0.0344	$9.8^{a\ A}$	$12.2^{a\ A}$	$8.9^{abc\ A}$	0.3414
BRM32114	$4.7^{ab\ B}$	$4.6^{c\ B}$	$5.1^{ab\ A}$	0.0471	$7.6^{a\ A}$	$6.8^{cd\ B}$	$7.2^{bc\ AB}$	0.0362
BRM32112	$4.6^{b\ B}$	$5.1^{bc\ A}$	$5.3^{ab\ A}$	0.0331	$7.6^{a\ A}$	$7.1^{bcd\ A}$	$11.1^{a\ A}$	0.0814
BRM32110	$4.6^{ab\ B}$	$5.4^{ab\ A}$	$5.7^{a\ A}$	0.0266	$9.9^{a\ A}$	$7.6^{bcd\ A}$	$8.6^{abc\ A}$	0.1015
BRM32109	$4.9^{ab\ A}$	$5.9^{a\ A}$	$4.9^{b\ A}$	0.8132	$8.7^{a\ A}$	$9.3^{bc\ A}$	$7.2^{bc\ A}$	0.2847
T. asperellum pool	$5.8^{a\ A}$	$5.0^{bc\ AB}$	$4.1^{c\ A}$	0.0365	$9.2^{a\ A}$	$9.4^{b\ A}$	$6.5^{c\ B}$	<0.001
ANOVA (*F* probability)	0.0471	<0.001	0.0396	–	0.1358	<0.001	0.0165	–
Microorganism	Mn content (mg kg^{-1})				Zn content (mg kg^{-1})			
BRM32113	$1528^{bc\ A}$	$1848^{b\ A}$	$1922^{a\ A}$	0.2879	$37^{b\ A}$	$40^{bc\ A}$	$49^{ab\ A}$	0.2418
BRM32111	$1950^{a\ B}$	$2848^{a\ A}$	$1772^{ab\ B}$	0.0214	$43^{ab\ A}$	$38^{c\ A}$	$42^{d\ A}$	0.3165
BRM32114	$1720^{abc\ A}$	$1512^{c\ B}$	$1764^{ab\ A}$	0.0308	$43^{ab\ B}$	$48^{ab\ A}$	$39^{d\ B}$	<0.001
BRM32112	$1643^{abc\ A}$	$1801^{b\ A}$	$1831^{ab\ A}$	0.6147	$38^{ab\ B}$	$42^{abc\ B}$	$49^{b\ A}$	<0.001
BRM32110	$1367^{c\ B}$	$1771^{b\ A}$	$1773^{ab\ A}$	<0.001	$49^{a\ A}$	$42^{abc\ B}$	$53^{a\ A}$	<0.001
BRM32109	$1491^{bc\ B}$	$1857^{b\ A}$	$1624^{b\ AB}$	0.0241	$42^{ab\ A}$	$42^{abc\ A}$	$47^{bc\ A}$	0.5719
T. asperellum pool	$1808^{ab\ A}$	$1691^{bc\ A}$	$1651^{b\ A}$	0.6792	$41^{ab\ A}$	$50^{a\ A}$	$43^{cd\ A}$	0.3201
ANOVA (*F* probability)	0.0268	0.0312	0.0415	–	0.0449	0.0197	<0.001	–

Means followed by the same letter, lowercase in column or upper case in line, do not differ by Tukey's test at $p \leq 0.05$

CV coefficient of variation

(photosynthesis, transpiration, and stomata conductance), dry matter accumulation, and nutrients to the water-soil-plant-atmosphere relationships (Auge 2001; Barzana et al. 2012).

Microorganisms can provide direct stimulus to plant growth by producing plant hormones or similar substances (Bandara et al. 2006; Guo et al. 2008; Pérez-García et al. 2011), or indirect stimulation by increasing the photosynthetic rate, as was shown in this study. Corroborating this information, the isolate BRM32114 (*Serratia* sp.) from the rhizosphere of rice plants (Table 1) improved the photosynthetic rate in 6.4% and the production of biomass to the order of 16.12%, considering all application forms and biomass in 19.14%, when it was applied only in seeds. Besides, this isolate was the only one that differed from the control in plant biomass production. Thus, the isolate BRM32114 (*Serratia* sp.) was considered the best microorganism to promote increases in rice shoot biomass in this research.

Regarding the nutritional status of the plants, it was observed that the plants treated with the isolate BRM32111 (*Pseudomonas* sp.), when applied to the seed, had the highest levels of N, P, Mn, and Fe, when compared to the other bioagents. However, these results may be related to the concentration effect (Fageria et al. 2011), since the plants treated with this isolate BRM32111 (*Pseudomonas* sp.) had the lowest biomass dry weight (24.11 g, Table 3). The isolate BRM32110 (*Bacillus* sp.), when only the seed was applied, was highlighted to increase contents of Cu and Zn in rice shoots, while the other *Bacillus* sp. isolate BRM32109, when seed-soil was applied, increased the levels of P and Ca in the rice shoots. Based on these results, it seems that microorganisms provided rice shoots an increased nutrient uptake. Microorganisms can also provide indirect plant growth stimulation by improving the availability and absorption of nutrients (Pérez-García et al. 2011; Zhang et al. 2011.). Thus, it can be inferred that, with the exception of K and Mg, most of the microorganisms studied during our research provided rice shoots a significant increase in nutrient content. These results suggest that both increased photosynthetic rate and an increase

in the content of nutrients in rice plants may have produced a direct effect on biomass production, which has a significant effect on a crop yield (Fageria 2009; Fageria et al. 2011).

Rêgo et al. (2014) reported that rice seeds treated with isolates *T. asperellum* pool, BRM32113 (*Burkholderia* sp.), and BRM32111 (*Pseudomonas* sp.) showed changes in root architecture by increasing the length of the roots, an expansion of the cortex, increased by 2% in aerenchyma spaces, an increase in diameter of the vascular cylinder, and an increase in the volume of the root and thereby confirmed that treated plants are more efficient in absorbing nutrients. Subsequently, Martins (2015) identified and characterized the genera of respective rhizobacteria (Table 1), indicating that the isolates BRM32113 (*Burkholderia* sp.) and BRM32114 (*Serratia* sp.) produce AAI, cellulase, and siderophores. In the same study, isolates BRM32111 (*Pseudomonas* sp.) and BRM32112 (*Pseudomonas* sp.) were identified and characterized, showing that they produce cellulose, phosphatase, and siderophores. This information helps to partly explain the higher biomass gain in plants treated with the isolate BRM32114 (*Serratia* sp.) (by seed application) and in plants treated with the isolate BRM32113 (*Burkholderia* sp.) (by seed-plant application). In addition, plants treated with the isolates BRM32111 and BRM32112 (both *Pseudomonas* sp.) (solubilizing phosphate) showed the highest levels of P and Fe.

Species of the genus *Trichoderma* are naturally found in almost all soil types and act against pathogens, using different action mechanisms; they also promote beneficial effects, such as plant growth (Shoresh et al. 2005; Perazzolli et al. 2008; Vinale et al. 2008). The *T. asperellum* pool used in this study is formed by isolated T-6, T-9, T-12, and T-52, which were selected and characterized by Silva et al. (2012), indicating that they are phosphate solubilizers and cellulose degraders and, in vitro, reduced the mycelial growth of *Rhizoctonia solani*.

During our research, we tested seven potential bioagents, which were previously identified as antagonists and inducers of resistance to the majority of rice pathogen (Filippi et al. 2011; Silva et al. 2012; Pereira Filho 2013). This paper presents the comparison of bioagents' beneficial effects in promoting the growth of rice as a novelty, which is done by assessing the health of the plant (gas exchange and nutritional status).

Based on the results, it appears that overall, the application form did not influence the results and then, only the application on the seeds could provide effects on the plant development. Besides, the use of bioagents can be a sustainable alternative for increasing the biomass production of rice plants, which is a characteristic that is positively correlated to a crop yield (Alvarez et al. 2012). This study also indicated that the isolates BRM32114 (*Serratia* sp.), the both *Bacillus* sp. BRM32110 and BRM32109, and the *T. asperellum* pool are the most promising to use on a commercial scale, since it excelled in promoting better performance of upland rice plants, besides being effective antagonists to the main rice pathogens (França et al. 2015; Souza et al. 2015; Martins 2015). Also, in general, these isolates provided higher nutrient concentration (Tables 4 and 5) in the rice plants, which indicates that their presence encourages the availability of nutrients in the soil and, consequently, the uptake of these by plants (Table 4). It is worth mentioning that the isolate BRM32114 (*Serratia* sp.) was the best bioagent, either to stimulate a higher photosynthetic rate (both in the vegetative and reproductive stage) or to promote greater accumulation of dry matter at 84 DAS in the rice plants, independent of application form, and because it was the only isolate that provided increases in the biomass production of rice that differed from the control (no microorganism application), further studies are needed to better understand the processes that are involved in bioagent-plant interaction, such as evaluating the efficiency of these bioagents under field conditions, quantifying key elements such as plant hormones and enzymes involved in physiological mechanism of growth induction and promoters of disease resistance, and defining the morpho-anatomical characteristics of the isolates during their interaction with the plant.

Conclusion

Overall, the application forms of microorganisms evaluated showed similar results. Then, we recommend only the application on the rice seeds. Microorganism application positively and differently affected the growth parameters evaluated in rice plants. Rice plants treated with isolates *Serratia* sp., *Bacillus* sp. (BRM32114, BRM32110, BRM32109), and *T. asperellum* pool provided, on average, the highest photosynthetic rate values and dry matter biomass of rice shoots. Plants treated with *Burkholderia* sp., *Serratia* sp., and *Pseudomonas* sp. isolates (BRM32113, BRM32114, BRM32111, and BRM32112) led to the greatest nutrient uptake by the rice shoots. However, it is complicated to use a mixture of microorganism under field conditions. Therefore, among the evaluated microorganisms, we selected the isolate BRM32114 (*Serratia* sp.) for future studies in field conditions, since it promoted, in rice shoots, the highest photosynthetic rate (both in vegetative and reproductive stage), for the greatest accumulation of nutrients and dry matter at 84 DAS, and for differing from the control in the characteristic of dry biomass production.

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