

Upland rice gas exchange, nutrient uptake and grain yield as affected by potassium fertilization and inoculation of the diazotrophic bacteria *Serratia* spp.

Adriano S. Nascente^{1*}, Marta Cristina C. Filippi¹, Thatyane P. Sousa², Amanda A. Chaibub³, Alan Carlos A. Souza⁴, Anna Cristina Lanna¹

¹Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Centro Nacional de Pesquisas de Arroz e Feijão, P.O. Box 179, Highway 462, km 12, Santo Antônio de Goiás, State of Goiás, 75.375-000, Brazil

²Escola de Agronomia, Universidade Federal de Goiás, Goiânia - GO, Brazil. 74.690-900

³Departamento de Fitopatologia, Universidade de Brasília, Brasília - DF, Brazil. 70.910-900

⁴Departamento de Fitopatologia, Universidade Federal de Lavras, Lavras - MG, Brazil. 37200-000

*Corresponding author: adriano.nascente@embrapa.br

Abstract

Potassium (K) is the second most uptaken nutrient by upland rice crops. Beneficial microorganisms, such as Rhizobacteria participate in different processes that affect transformation of soil nutrients, making them available to plants. However, there are no information about the use of Rhizobacteria and different rates of K fertilization in this crop. To elucidate this interaction, the objective of this study was to determine the effect of the rhizobacteria *Serratia* spp. strain BRM 32114 and doses of K at sowing fertilization on upland rice development. The experimental design was a complete randomized block in a factorial 4 × 2 scheme with four replications, under no-tillage systems conducted in the Brazilian Cerrado during 2015/16 and 2016/17 growing seasons. The treatments consisted of four K₂O doses (0, 20, 40 and 60 kg ha⁻¹) without or with rhizobacteria applied three times in the field (on the seeds, sprayed over the soil, seven days after sowing and sprayed on the plants, 14 days after sowing). Biomass production, gas exchange, nutrient content in leaves and grain, yield components and grain yield were evaluated. The use of BRM 32114 applied in soil with high levels of K provided increase in the contents of P, Ca, Mg, Fe and Zn in rice leaves. The results showed enhances in plant biomass (7.2%), the number of panicles per plant (10%), and the grain yield of upland rice (16.3%). Therefore, our results allow inferring that the use of microorganism BRM 32114 provided significant improvements in rice growth/development, which resulted in higher grain yield over two consecutive growing seasons under field conditions. It showed that the bioagent *Serratia* spp. is promising to be incorporated into crop systems.

Keywords: *Oryza sativa*; bioagent; rhizobacteria; fertilizer; growth promoter; sustainable development.

Abbreviations: PBRM_Plant Beneficial Rhizospheric Microorganisms, IAA_indole acetic acid, A_photosynthesis, E_transpiration, gs_stomata conductance, PGPR_Plant Growth-Promoting Bacteria, Ci_internal CO₂ concentration, Tleaf_leaf temperature, IRGA_Infra Red Gas Analyzer, PPFD_Photon Flux Density Photosynthetic Active.

Introduction

Rice is one of the most important food crops in the world with a global per capita consumption around 57 kg yr⁻¹. Therefore, the increase in total consumption should follow the rate of population growth (Chakraborty et al., 2017). It is cultivated in 159 million hectares (Mha), representing 11% of cultivated area, with an annual production of 703.8 million tons (Mt) of paddy. From the total area, 6 million ha is cultivated with upland, 9% in Asia, 46 % of Latin America including the Caribbean countries, and 47 % of West Africa (Kikuta et al., 2016).

Agriculture sustainability can be defined as the common face of agronomic, ecologic, economic and social factors. However, food production in sustainable systems faces the challenge of increasing fertilizer application so that the productivity limit can be advanced. Also, the integrity of the

environment must be preserved, protecting ecosystems and biodiversity (Meena et al., 2017).

There are many studies showing significant increase in rice grain yield due to K fertilization (Fageria et al., 1990; Fageria, 2000; Farinelli et al., 2004; Carmeis Filho et al., 2017). For upland rice plants, K is essential for growth and development. It is extracted from the soils in large amounts, such as 245 kg ha⁻¹ (Slaton et al., 2009; Fageria et al., 2010; Fageria et al., 2011b). The principal roles of K in plants are mainly the osmotic potential regulation, acting in the transporters mechanism in the cell membrane of root tissues, increasing root development in rice (Fageria et al., 2010; Fageria et al., 2011a; Clover and Mallarino, 2013) and accumulating in the shoots (Crusciol et al., 2016).

Although great amounts of K is required, there is a wish to increase crop grain yield with a significant reduction of

fertilizer and pesticides (Bashan et al., 2014), once this reduction is environmentally friendly and yield efficient.

In this sense, adopting Plant Beneficial Rhizospheric Microorganisms (PBRM) is one practice that can reduce the negative environmental impact caused by excess chemical fertilizers and agrochemicals, playing an important role in sustainable agriculture (Vejan et al., 2016). In microflora, PBRMs 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, or solubilizing phosphorus and producing siderophores (Spaepen et al. 2009; Ahemad and Kilbret 2014). Among the indirect benefits is the protection against pathogens, such as the induction of resistance and direct antagonism.

These bacteria 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) and *Pseudomonas* (Yao et al., 2010), *Bacillus*, *Serratia*, *Paenibacillum*, *Enterobacter* and *Klebsiella* (Spaepen et al., 2009).

Research focusing on the use of rhizobacteria in rice crops selected promising rhizobacteria isolates which stood out mainly due to their increased biomass production and disease resistance (Filippi et al. 2011; Silva et al. 2012; França et al. 2015). These rhizobacteria was tested under greenhouse conditions and showed that *Serratia* ssp, strain BRM 32114, promoted gas exchange, nutrients accumulation and biomass production, which differed from the control (untreated plants) in upland rice plants (Nascente et al., 2017). Effects of this rhizobacteria can be affected by mineral nutrition of the plants (Ahemad and Kilbret, 2014). However, there is still no information combining upland rice plant, rhizobacteria and potassium fertilization under field conditions of a Tropical region. Therefore, the objective of this study was to determine the effect of the rhizobacteria *Serratia* spp., strain BRM 3214, and doses of K at sowing fertilization on upland rice by biomass production, gas exchange, nutrient content in leaves and grain, yield components and grain yield under no-tillage systems in a Tropical Region.

Results

Gas exchange of rice plants as affected by rhizobacteria

There was no difference in gas exchange parameters for upland rice plants grown in the presence and absence of *Serratia* spp. (strain BRM32114) and at different K levels (Table 2). For photosynthetic rate (A), there was interaction between BRM32114 and evaluation time, growing season and evaluation time with growing season (Table 3). Thus, BRM32114-treated upland rice presented greater A and E at the reproductive stage in growing season 2015/16 and

higher A, E and gs at the vegetative phase in growing season 2016/17.

Nutrient content in rice leaves

There was single effect of BRM32114 on N and Fe content in upland rice leaves (Table 4). Nitrogen content was increased in no treated plants (without BRM 32114); while Fe content increased in BRM 32114-treated plants. There was interaction between BRM32114 and growing season for P, Ca, Mg and Zn content in leaves of upland rice. So, in 2015/16, the Zn content was higher in BRM 32114-treated plants compared to no treated plants. In 2016/17, BRM 32114-treated plants presented higher values of P, Ca and Mg content. There was interaction between K rates and growing season for K content in rice leaves (Fig. 2).

Plant biomass, yield components and grain yield of upland rice plants

The use of BRM32114 provided increases in plant biomass, number of panicle per plant and grain yield of upland rice (Table 5). Number of grains per panicle and mass of 1000 grains of upland rice plants were not affected by use of BRM 32114. The K levels had no effect on these parameters.

Regarding growing season effect, in 2016/17, plants presented higher biomass, number of panicle per plant, number of grains per panicle and grain yield than 2015/16. BRM 32114-treated rice yield was 4921 kg ha⁻¹ compared to 3983 kg ha⁻¹ in no treated plants at the growing season 2016/17. For the growing season 2015/16, these values were 2663 kg ha⁻¹ with BRM 32114 and 2363 kg ha⁻¹ without BRM 32114 (Table 5).

Nutrient content in rice grains

Untreated plants presented higher N content in rice grains (Table 6). In growing season 2015/16, the contents of P, K, Ca, Mg and Fe in grains were higher in BRM 32114-treated plants. There was interaction between BRM32114 and growing season for Cu, Mn and Zn, being the highest content of them observed in the grains of no treated plants, in 2016/2017.

Discussion

Researches at Embrapa Rice and Beans found that application of on *Serratia* spp. (a gram negative anaerobic bacteria) strain BRM 32114, produces IAA, cellulase and siderophores, which enhance resistance, photosynthetic rate, root development, dry matter and uptake/accumulation nutrients in rice shoots, (Nascente et al., 2017; Sperandio et al., 2017). In our trials, we describe an exceptional grain yield increase promoted by BRM32114 under field conditions independent from K doses applied. Our results show that K rates (20, 40 and 60 kg ha⁻¹) presented no effect on the evaluated parameters. This is probably because in both growing seasons the K content in the soil was very high (101,3 mg dm⁻³ and 97 mg dm⁻³ in 2015/16 and 2016/17, respectively) (Table 1) (Sousa and Lobato, 2004). So, the soil with 0 kg ha⁻¹ of K did not show any deficiency or unbalanced evidence. Corroborating this information, Zaratin et al. (2004) observed that K fertilization

Table 1. Chemical and physical properties of the soil at the beginning of the trial.

Growing season 2015/2016							
Depth (m)	Ca	Mg	H+Al	SOM ¹	P	K	pH (H ₂ O)
0-0.20	18.0	13.3	24.0	32.7	9.9	101.3	5.9
Growing season 2016/2017							
Depth (m)	Ca	Mg	H+Al	SOM ¹	P	K	pH (H ₂ O)
0-0.20	10.7	10.9	31.0	42.22	12.6	97	5.5
Depth (m)	Cu	Zn	Fe	Mn	Clay	Silt	Sand
0-0.20	0.8	4.6	20.5	10.1	480	226	294
Depth (m)	Cu	Zn	Fe	Mn	Clay	Silt	Sand
0-0.20	1.2	8.1	31.0	12.2	520	180	300

¹SOM – soil organic matter.

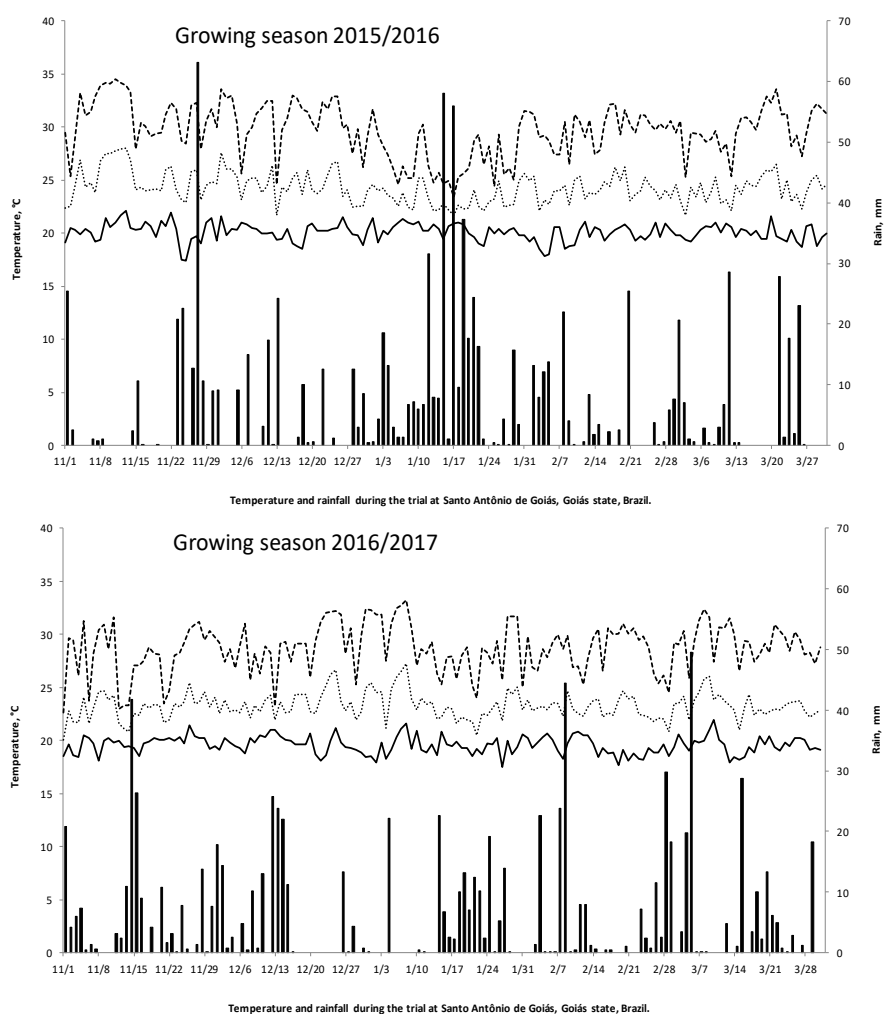


Fig 1. Maximum (T maximum), minimum (T minimum) and average (T average) temperatures and rainfall during the trial period of upland rice grown under a no-tillage system in the experimental fields of Santo Antônio de Goiás in the 2015/16 and 2016/17 growing seasons.

Table 2. Potassium rates and *Serratia* spp. isolated BRM 321114 with or without inoculation in rice seeds as affecting photosynthesis (A), transpiration (E), stomatal conductance (gs), internal CO₂ concentration (Ci) and leaf temperature (Tleaf) of rice plants in two evaluation times at vegetative and reproductive phase of rice development. Santo Antônio de Goiás, growing season 2015/2016 and 2016/2017.

Factors	A	E	gs	Ci	Tleaf
<i>Serratia</i>	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$	$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$	$\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	vpm	°C
With	25.03 ^{ns}	6.00 ^{ns}	1.01 ^{ns}	288 ^{ns}	30.00 ^{ns}
Without	24.82	5.95	1.00	289	29.81
<u>Evaluation time</u>					
Vegetative	26.42 a	6.27 a	1.07 a	296 a	29.33 b
Reproductive	23.42 b	5.69 b	0.94 b	282 b	30.59 a
<u>Growing season</u>					
2015/2016	22.61 b	5.15 b	0.56 b	268 b	31.10 a
2016/2017	27.24 a	6.80 a	1.45 a	310 a	28.71 b
<u>Factors</u> ANOVA (F Probability)					
<i>Serratia</i> (S)	0.4807	0.7935	0.9190	0.7163	0.5054
Potassium rates (K)	0.4579	0.9141	0.3771	0.3340	0.9771
Evaluation time (ET)	<0.001	0.0010	0.0129	<0.001	<0.001
Growing season (GS)	<0.001	<0.001	<0.001	<0.001	<0.001
S x K	0.0510	0.6015	0.1839	0.4103	0.8614
S x ET	0.0268	0.1394	0.1895	0.3278	0.0137
S x GS	0.0040	0.0517	0.8881	0.0111	0.7984
K x ET	0.0599	0.5199	0.9407	0.0509	0.9646
K x GS	0.1055	0.5364	0.4961	0.0503	0.9822
ET x GS	<0.001	<0.001	0.0028	0.0617	0.1510
S x K x ET	0.6719	0.5196	0.4750	0.1421	0.9313
S x K x GS	0.2986	0.5923	0.3448	0.1008	0.8021
K x ET x GS	0.0523	0.8752	0.5022	0.6208	0.8010
S x ET x GS	0.3431	0.2176	0.5323	0.1742	0.0520
S x ET x GS x K	0.3126	0.3715	0.5290	0.1710	0.8877

* Means followed by the same letter do not differ by Tukey test. ^{ns} Means no significance.

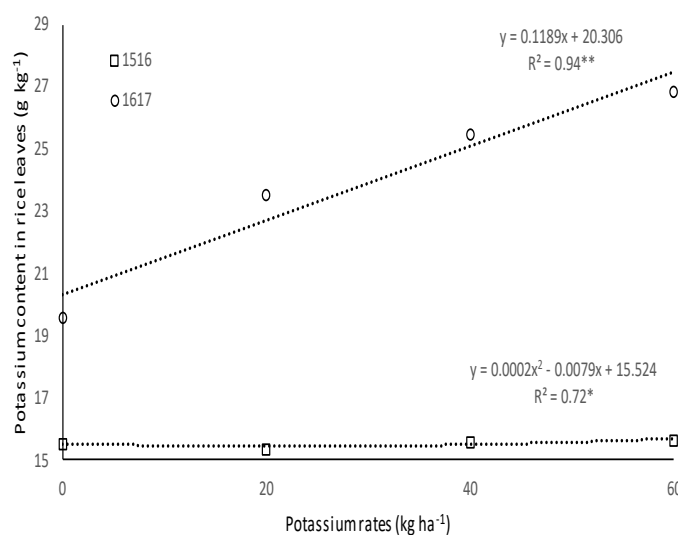


Fig 2. Potassium content in rice leaves as a function of potassium rates and growing season. Santo Antonio de Goias, GO, growing seasons 2015/16 e 2016/17.

Table 3. Interaction between growing season and evaluation time for photosynthesis (A), transpiration (E), internal CO₂ concentration (Ci) and leaf temperature (Tleaf) of rice plants at vegetative and reproductive phase of rice development, and with or without the use of Rhizobacterium with transpiration, stomatal conductance (gs), internal CO₂ concentration and leaf temperature of upland rice plants. Santo Antônio de Goiás, growing season 2015/16 and 2016/17.

Interaction between Rhizobacterium and evaluation time			
Vegetative			
Rhizobacterium	A μmol CO ₂ m ⁻² s ⁻¹	E mmol H ₂ O m ⁻² s ⁻¹	Tleaf °C
With	26.19 ^{ns}		29.69 a
Without	26.66		28.75 b
Reproductive			
Rhizobacterium			
With	23.88 a		30.32 b
Without	22.97 b		30.86 a
Interaction between growing season and evaluation time			
Growing season 2015/16			
Evaluation time	A μmol CO ₂ m ⁻² s ⁻¹	E mmol H ₂ O m ⁻² s ⁻¹	gs mol H ₂ O m ⁻² s ⁻¹
Vegetative	21.36 b	4.86 b	0.54 ^{ns}
Reproductive	23.85 a	5.44 a	0.57
Growing season 2016/2017			
Evaluation time			
Vegetative	31.48 a	7.67 a	1.60 a
Reproductive	23.00 b	5.93 b	1.30 b
Interaction between growing season and Rhizobacterium			
Growing season 2015/16			
Rhizobacterium	A μmol CO ₂ m ⁻² s ⁻¹	Ci vpm	
With	23.17 a	271 ^{ns}	
Whiteout	22.05 b	265	
Growing season 2016/17			
Rhizobacterium			
With	26.90 ^{ns}	306 b	
Without	27.59	314 a	

* Means followed by the same letter do not differ by Tukey test. ^{ns} Means no significance.

Table 4. Potassium rates and *Serratia* spp. isolated BRM 321114 with or without inoculation in rice seeds as affecting nutrient content in rice leaves at full flowering stage. Santo Antônio de Goiás, growing season 2015/2016 and 2016/2017.

Factors	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
<i>Serratia</i>			g kg ⁻¹				mg kg ⁻¹		
With	20.71 b	3.18 ^{ns}	20.23	3.48 a	2.42 ^{ns}	6.86 ^{ns}	130 a	75 ^{ns}	43 a
Without	22.11 a	3.29	19.18	3.31 b	2.40	6.31	121 b	71	40 b
Growing season									
2015/2016	12.36 b	4.18 a	15.53 b	2.35 b	2.70 a	6.33 ^{ns}	185 a	56 b	64 a
2016/2017	30.46 a	2.29 b	23.87 a	4.43 a	2.11 b	6.84	67 b	89 a	19 b
ANOVA (F probability)									
<i>Serratia</i> (S)	<0.001	0.4648	0.2107	0.0443	0.7086	0.1795	0.0479	0.4100	0.0495
Potassium rates (K)	0.2081	0.3196	0.0169	0.9365	0.8217	0.4565	0.5427	0.7443	0.2383
Growing season (GS)	<0.001	<0.001	<0.001	<0.001	<0.001	0.2113	<0.001	<0.001	<0.001
S x K	0.2055	0.6517	0.5475	0.1063	0.3970	0.3042	0.0631	0.1555	0.4371
S x GS	0.1145	0.0434	0.0914	0.0027	0.0043	0.6506	0.7487	0.2989	0.0360
K x GS	0.1606	0.5236	0.0212	0.2964	0.8417	0.5555	0.5942	0.5480	0.2193
S x K x GS	0.1910	0.4421	0.8057	0.5583	0.8890	0.5369	0.7774	0.6360	0.6022
Interaction between Rhizobacterium and growing season									
Rhizobacterium	-----Growing season 2015/2016-----				-----Growing season 2016/2017-----				
	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	mg kg ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
With	3.98 ^{ns}	2.29 ^{ns}	2.63 ^{ns}	67.22 a	2.37 a	4.66 a	2.21 a	18.30 ^{ns}	
Without	4.38	2.42	2.78	60.52 b	2.20 b	4.20 b	2.02 b	18.92	

* Means followed by the same letter do not differ by Tukey test at p<0.05. ^{ns} Means no significance.

Table 5. Potassium rates and *Serratia* spp. isolated BRM 321114 with or without inoculation in rice seeds as affecting plant biomass (PB), number of panicles per plant (NPP), number of grain per panicle (NGP), mass of 1000 grains (1000M) and grain yield (GY) of upland rice. Santo Antônio de Goiás, growing season 2015/2016 and 2016/2017.

Factors	PB	NPP	NGP	1000M	GY
<i>Serratia</i>	g m ⁻²	unit	unit	g	kg ha ⁻¹
With	222 a	81 a	121 ^{ns}	23.17 ^{ns}	3792 a
Without	206 b	73 b	124	22.98	3174 b
Growing season					
2015/2016	186 b	76 ^{ns}	116 b	23.06 ^{ns}	2513 b
2016/2017	243 a	78	130 a	23.09	4453 a
ANOVA (F probability)					
Serratia (S)	0.0452	0.0068	0.5083	0.5017	<0.001
Potassium rates (K)	0.8374	0.0890	0.5497	0.6562	0.1255
Growing season (GS)	0.0100	0.5007	0.0076	0.9105	<0.001
S x P	0.3043	0.6678	0.7729	0.2238	0.5159
S x GS	0.3704	0.9615	0.5002	0.6006	0.0157
P x GS	0.5821	0.7061	0.3312	0.3995	0.8676
S x P x GS	0.5350	0.2487	0.9572	0.1096	0.3350
Interaction between growing season and Rhizobacterium					
	Growing season 2015/16		Growing season 2016/17		
<i>Rhizobacterium</i>	-----Grain yield kg ha ⁻¹ -----				
With	2663 a		4921 a		
Without	2363 b		3983 b		

* Means followed by the same letter do not differ by Tukey test. ^{ns} Means no significance.

Table 6. Potassium rates and *Serratia* spp., strain BRM 32114, with or without inoculation in rice seeds as affecting nutrient content in rice grains. Santo Antônio de Goiás, growing season 2015/2016 and 2016/2017.

Factors	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
<i>Serratia</i> R-235	-----g kg ⁻¹ -----								
With	13.18 b	3.29 ^{ns}	2.61 ^{ns}	0.32 ^{ns}	1.47 ^{ns}	5.07 ^{ns}	28.10 ^{ns}	21.07 ^{ns}	34.99 ^{ns}
Without	14.09 a	3.25	2.58	0.32	1.47	5.11	31.16	22.56	35.64
Growing season									
2015/2016	13.39 ^{ns}	3.71 a	2.93 a	0.33 a	1.59 a	5.46 ^{ns}	31.51 a	19.10 ^{ns}	37.28 ^{ns}
2016/2017	13.96	2.69 b	2.14 b	0.31 b	1.31 b	4.59	27.13 b	25.44	32.69
ANOVA (F probability)									
<i>Serratia</i> R-235 (S)	0.0294	0.4045	0.3053	0.3360	0.9665	0.3943	0.1515	0.1521	0.1813
Potassium rates (K)	0.1104	0.3850	0.3296	0.1057	0.1026	0.9414	0.3623	0.4670	0.2235
Growing season (GS)	0.1073	<0.001	<0.001	<0.001	<0.001	<0.001	0.0097	<0.001	<0.001
S x K	0.4771	0.9171	0.4529	0.2675	0.2094	0.5952	0.5686	0.1487	0.8679
S x GS	0.4176	0.4712	0.7910	0.8088	0.2603	0.0070	0.1632	0.0415	0.0116
K x GS	0.0885	0.3850	0.7483	0.0556	0.7472	0.4675	0.3333	0.7475	0.4088
S x K x GS	0.3713	0.1371	0.4458	0.0519	0.7780	0.8341	0.4064	0.9635	0.8826
Interactions between Rhizobacterium and growing season									
	Growing season 2015/2016			Growing season 2016/2017					
<i>Rhizobacterium</i>	-----mg kg ⁻¹ -----								
With	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Without	5.53	19.52	37.70	4.47 b	23.16 b	31.38 b	4.72 a	27.73 a	34.01 a

* Means followed by the same letter do not differ by Tukey test. ^{ns} Means no significance.

increased the yield components of rice grains in Cerrado Oxisol with K content of 82 mg dm⁻³ in the soil. This value is 20% lower than the values found in our areas.

We believe that the presence of BRM 321114 with the high K contents in the soil provided nutrients and increased nutrient uptake ability of rice plants, especially of Zn (growing season 2015/16), P, Ca, and Mg (growing season 2016/17). According to Medeiros et al. (2005), plants with well-developed root systems have the ability to exploit a greater soil volume, which is fundamental for increasing the

contact between roots and nutrients, resulting in an improvement in nutrient uptake.

We report that the treatment of upland rice with BRM 32114 was responsible for the positive changes observed in photosynthetic and transpiratory rates (A and E, respectively), stomata conductance (gs), internal CO₂ concentration) during the vegetative stage (Table 2). In turn, it is also responsible for the increase in yield components (Table 4). We believe these are the result of a specific and direct interaction, generally or indirectly, or both at the same time (Pérez-Montaño et al., 2014).

As direct effects, we know that BRM 32114 produces phytohormones, siderophores and solubilizes phosphate, as reported by Sperandio et al. (2017). These direct effects may have been responsible for the increase in the growth rate of the plants. Consequently, as an indirect effect the positive changes in the gaseous exchanges of upland rice may have been due to the supply of various nutrients to the plant, enhancing the metabolism. Some enzymes such as lipases and proteases produced by beneficial bacteria in the soil have many positive roles upon release. They can provide several nutritional substances for rice plants that increase metabolism (Sperandio et al., 2017; Nascente et al., 2017; Bandara et al., 2006). Hence, they can improve the overall biological health of the soil (Guo et al., 2008; Cassán et al., 2009; Mei and Flinn, 2010; Pérez-García et al., 2014). Sperandio et al. (2017) reported that the interaction between upland rice plants and BRM 32114 has increased the root and shoot lengths by 41.9% and 43.4%. The same interaction has increased the root, shoot biomass and foliar area up to 46.5%, 70% and 23.64%, respectively.

Our data shows that treatment with BRM 32114 changed basic metabolism such as photosynthesis no matter how much K was available during rice cycle. Although, to deepen and clarify the understanding knowledge on the interaction between plant and beneficial bacteria we must investigate the mechanisms used by BRM 32114, when interacting with the plant.

Along with that, higher nutrient uptake could explain in part the higher grain yield in 2016/17. For example, in 2016/17 the content of K in rice leaves was much higher than in 2015/16 (Fig. 2). According to Fageria (2014), a plant that grows more vigorously and consequently has higher grain yield, usually absorb more nutrients and raise their content in the leaves. On the other hand, it was observed that the higher availability of nutrients due to the presence of BRM 32114 affected the nutritional status of the shoot, but not the upland rice grain quality, with exception of N. Besides, in the growing season 2016/2017, the experimental field has a more fertile soil (Table 1 and Sousa and Lobato, 2004) and rainfall distribution was more appropriate, since in the period from flowering to grain filling there was more rain (370.0 mm) than in the growing season 2015/2016 (195.4 mm).

For N contents in both growing season and for Cu, Mn and Zn in the growing season 2016/17, no treated plants showed higher values of these nutrients in rice grains than in growing season 2015/16. This fact could be related to the concentration effect (Fageria et al., 2011b), once in this season, the grain yield of no treated plants was significantly lower than treated plants (Table 4).

Upland rice plants with higher nutrients content reflected directly on biomass accumulation (7.2%) and crop yield (16.3%). Nascente et al. (2017), worked with the same cultivar and bacterial isolates and described an increase of 16.1% in rice biomass production. According to Alvarez et al. (2012), rice biomass production of plants positively correlated with crop grain yield.

Many species classified as PGPRs have been studied in order to be included in cropping systems. So, many of these PGPRs have already been commercialized, such as the genus *Pseudomonas*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Azobacter*, *Variovorax*, *Azospirillum*, and *Serratia* (Glick, 2012). Our research seems promising and showed that using

biodiversity (microorganism collected from the rhizosphere of upland rice) could bring many benefits to the crops systems. In this sense, using upland rice treated with beneficial microorganism could contribute to improve the profitability of the crop for the farmers, since the tested rhizobacterium provided advantages to the plant as an adequate profile of gas exchanges and availability of nutrients, independent from high K level in the soil. The use of microbial for integrated nutrient management of rice production can be a sustainable way of agriculture (Lavakush et al., 2014). It turns to be an environmental friendly alternative to chemical fertilizer for upland rice production as well as seems to enhance the soil fertility and health.

Materials and methods

Site description

The experiments were conducted at Capivara Experimental Station of Embrapa Rice and Beans, located at Santo Antônio de Goiás, GO, Brazil, 16°28'00"S and 49°17'00"W coordinates, and at 823 m of elevation. The climate is Tropical Savanna and is considered Aw according to its Köppen classification. There are two well-defined seasons: a usually dry season from May to September (autumn/winter) and a wet season from October to April (spring/summer). The average annual rainfall is between 1500 and 1700 mm, and the average annual temperature is 22.7°C, ranging annually from 14.2 °C to 34.8 °C. During the period of this study, the temperature and the amount of rainfall data were recorded (Fig. 1).

The experimental soil is classified as a clay loam (kaolinitic, thermic Typic Haplorthox) acidic soil (Embrapa, 2006). Prior to the study, in November of 2015 and 2016, chemical and physical analyses were performed according to the methodology by Donagema et al. (2011) for the initial characterization of the area (Table 1).

Experimental area had been cultivated in a program of crop rotation, named as crop-livestock integration, in a no-tillage system for five consecutive years, composed of soybean (summer, year 1), rice (summer, year 2) and irrigated common bean (winter, year 2), corn + *Brachiaria* (summer, year 3), and two years (years 4 and 5) of grazing pasture. The arrangement of experiments was conducted in plots where the upland rice was the crop to be grown following the established.

Experimental design and treatments

Trials were conducted in rainfed conditions using the cultivar 07SEQCL441 CL (resistant to the Imazapyr + Imazapic herbicide). Trials were arranged in a complete randomized block in a 4x2 factorial scheme with four replications, during two growing seasons (2015/16 and 2016/17) in different areas. The plots had the dimension of 3.5 m x 6 m. The usable area of the plot was composed of the four central rows of rice, disregarding one row of each side of the plot and 0.50 m to each side of the rows in each plot. The treatments consisted of combining four broadcasted K₂O doses (0, 20, 40 and 60 kg ha⁻¹ as potassium chloride) at the time of sowing with or without BRM 32114 applied on seed (microbialization) and sprayed at plant/soil in the field at the 7 and 15 DAS (days after sowing). BRM 32114 isolate

belongs to the Microorganism Culture Collection at the Embrapa Rice and Bean Research Center, with KX378746 GenBank code.

Seed microbialization and plant/soil spray pulverization

BRM 32114 bacterial suspension was prepared with water using the cultures that had been growing for a 24-hour period on solid medium 523 (Kado and Heskett, 1970), at 28 °C, and the concentration was set in a spectrophotometer to $A_{540} = 0.5$ (10^8 UFC). Rice seeds were immersed in the bacterial suspension for 24 hours at 25 °C under constant agitation. The control seeds (no bacteria) were immersed only in water for 24 hours in the same conditions. Soil pulverization was done at 7 days and plant pulverization at 15 days after rice sowing in all plots with rhizobacteria, with bacterial suspension prepared as described above, in the same concentration (10^8 UFC), at 600 l ha^{-1} , with a costal atomizer.

Rice crop management

Cover crops were desiccated with a glyphosate application (1.8 kg ha^{-1} acid equivalent) 30 days before sowing of the upland rice. The sowing was performed mechanically using 200 seeds per m^2 of rice. The seeds were sown on December 15th, 2015 and on November 23rd, 2016. Seven days before the sowing of the rice, the seed microbialization was carried out. Rice plant emergence was occurred five days after sowing for both growing seasons. The base fertilization was applied in the sowing furrows. It was calculated according to the soil's chemical characteristics and followed the recommendations of Sousa and Lobato (2003). Therefore, the sowing fertilization was 104 kg ha^{-1} of P_2O_5 + 18 kg ha^{-1} of N as monophosphate ammonium. The topdressing fertilization was performed 18 days after sowing using 45 kg ha^{-1} of N as urea in both years. Cultural practices were performed according to standard recommendations for a rice crop to keep the area free from weeds, diseases and insects.

Gas exchange in rice plants

The gas exchange was sampled from the rice plants at the vegetative and reproductive stages. Measurements were carried out using a portable gas exchange analyzer in the infrared region (LCpro+, ADC BioScientific). The parameters measured were: photosynthesis (A, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), internal CO_2 concentration (C_i , ppm) and leaf temperature (T_{leaf} , °C). The readings were recorded from 08:00 to 10:00 AM, at 37 and 68 DAE for the first growing season and 49 and 79 DAE for the second growing season.

Samples were taken in the middle third of the first fully expanded leaf (top to base) during the two evaluation periods in the two-year experiment. The equipment was set to use concentrations of $370\text{--}400 \text{ mol mol}^{-1} \text{ CO}_2$ in the air, which is the reference condition used in the IRGA photosynthesis chamber. The photon flux density photosynthetic active (PPFD) was $1200 \mu\text{mol [quanta] m}^{-2} \text{ s}^{-1}$. The minimum equilibration time set for performing the reading was 2 minutes.

Nutrients in leaves and grains of rice

Rice leaf samples were collected from the flag leaf at the full blooming stage. Leaves from 50 plants per plot were collected as proposed by Malavolta (2006), washed and then dried under forced-air circulation at 65 °C for 72 h before grinding and analyzing the samples for chemical composition. Besides, in each plot, one sample of 100 g of grains was taken for nutrient analyses. In leaves and grains, the concentrations of N, P, K, Ca, Mg, Cu, Zn, Fe and Mn were determined using methods described by Malavolta et al. (1997).

Shoot dry biomass of rice plants

Rice shoots of 1.0 m from one of the rows, in each plot were sampled at the full blooming stage. The shoots were washed in water and then dried in an oven with forced air circulation at 65 °C, then weighed to determine the shoot dry mass.

Yield components and grain yield of rice

Rice harvest was carried out by hand after physiological maturity (March 30th, 2016 and March 21st, 2017) of the grains in the usable area of each plot. Plots were evaluated for the number of panicles m^{-1} , which was determined by counting the number of panicles within 1.0 m of one of the rows in the useful area of each plot; number of grains per panicle, which was determined by counting the number of grains in 10 panicles random sampled in the usable area and divided by 10; the mass of 1000 grains, which was randomly evaluated by collecting and weighing 1000 grains from each plot, corrected to 13% of water content; and the grain yield, which was determined by weighing the harvested grain of each plot, corrected to 13% of the water content and converted to kg ha^{-1} .

Statistical analysis

For statistical analysis, the SAS Statistical Software, SAS Institute, Cary, NC, USA (SAS, 1999) was used. For the qualitative factor (with or without BRM 32114), data was subjected to an analysis of variance, and when the F test proved significant, the data was compared using a Tukey test at $p < 0.05$. In the quantitative factor (K rates), results were submitted to regression analysis (when they were $p < 0.05$). The blocks and all block interactions were considered as random effects. The K rates, use of rhizobacteria (BRM 32114) and year were considered as fixed effects.

Conclusion

The use of Rhizobacterium *Serratia* spp., strain BRM 32114, in the soil with high level of K, increased the content of P, Ca, Mg, Fe and Zn in rice leaves, plant biomass, number of panicles per plant, number of grains per panicle and grain yield of upland rice. Grain yield of rice treated with Rhizobacterium *Serratia* spp. isolate BRM 32114 was 16.3% higher than no treated plants. The use of bioagent was effective to provide better plant upland rice growth/development under field conditions and showed promising to be incorporated into crop systems.

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