Vol. 16(6), pp. 765-776, June, 2020
DOI: 10.5897/AJAR2020.14723
Article Number: D0AFA7163813
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

ACADEMIC JOURNALS
expand your knowledge

African Journal of Agricultural Research

Full Length Research Paper

Promotion of rice growth and productivity as a result of seed inoculation with *Azospirillum brasilense*

Vandeir Francisco Guimarães[1]*, Jeferson Klein[2], Marcos Barbosa Ferreira[3] and Débora Kestring Klein[2]

[1]Bolsista de Produtividade CNPq, Agricultural Sciences Center, Parana Universidade Estadual do Oeste do Paraná – Unioeste, Pernambuco street, 1777, Box 91, Zip Code: 85960-000, Marechal Cândido Rondon - PR, Brazil.
[2]Biogenesis Centro de Pesquisa e Desenvolvimento, Toledo, PR, Brazil.
[3]Mestrado Profissional em Produção e Gestão Agroindustrial - Universidade Anhanguera-Uniderp, Campo Grande/MS, Brazil.

Received 15 January, 2020; Accepted 13 March, 2020

The present study refers to the results obtained in four experiments carried out in the 2015/2016 crop: Toledo/PR; Palotina/PR; Cascavel/PR; and São Miguel do Iguaçu/PR. The objective of the present study was to evaluate the agronomic efficiency of *Azospirillum brasilense* (pest strains Ab-V5 and Ab-V6) in four distinct rice culture regions (*Oryza sativa* L.). The tests were performed to meet the requirements of the Ministry of Agriculture, Livestock and Supply regarding the registration of the product. The experiments were conducted in a randomized block design with seven treatments and four replications, totaling 28 plots each. Rice cultivar IAC 201 was used in all four experiments. At stage R7, 10 plants were collected to measure morphobiometric variables and nitrogen content in grains (N grains). The results showed that in all evaluated sites the use of liquid inoculation with *A. brasilense* increased rice crop development as well as productivity. Thus, inoculation of rice seeds with *A. brasilense* strains Ab-V5 and Ab-V6 based inoculant in the liquid formulations increased the yield and yield components of rice crop in different regions. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6, using the liquid vehicle, presented higher agronomic efficiency for rice cultivation. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the peat medium has good agronomic efficiency similar to the standard inoculant. These results were satisfactory contributing also to the sustainability in the agriculture.

Key words: Diazotrophic bacteria, plant growth promoting bacteria, nitrogen fertilization, seed inoculation, *Oryza sativa L*.

INTRODUCTION

Rice (*Oryza sativa* L.) is a cereal that is present daily in the diet of much of the world's population, being one of the most widespread crops in the world (Areias et al., 2006). Estimated world production for grain since 2017 is

*Corresponding author. E-mail: vandeirfg@yahoo.com.br.

over 475 million tons, of which 8.3 million tons are produced in Brazil (USDA, 2017). In relation to the world ranking in recent years, Brazil is always among the top ten producers of *O. sativa* compared to Latin American countries (CONAB, 2016). These results are important because there is a small gradual reduction in the country over the same period of rice acreage. However, in this scenario, the state of Paraná maintains its prominence, as it does not present large production fluctuations, currently guaranteeing the 7th position in the national ranking of producers of this crop.

It is well known to the scientific community that there are several factors that can interfere with rice crop productivity, among them nitrogen fertilization plays an important role (Hernandes et al., 2010). Mainly due to the nutritional sources required for this crop which come from the mineralization of soil nitrogen (Vogel et al., 2013). Of all the chemical elements, nitrogen undoubtedly deserves prominence in the development of any crop, mainly because it directly influences the growth of plants thus enhancing their production (Malavolta and Moraes, 2007). In relation to rice cultivation, this chemical element may contribute to the increase in the number of tillers and, thus, also increase the number of plant panicles, besides promoting greater number of spikelet grenades and higher protein content in grains (Rojas et al., 2012). However, despite the importance of this mineral, its use for a good yield of *O. sativa* production is dependent on high nitrogen doses. However, their excess can cause problems caused by self-shading or bedding, factors that may also provide adequate conditions for the creation of favorable conditions for disease occurrence (Neves et al., 2004).

Since the early nineties, several researchers have directed their investigations to increase the efficiency of biological nitrogen fixation performed by plant growth promoting bacteria in Brazil (Dobereiner, 1990).

In the literature it is possible to observe different beneficial effects caused by a wide list of micro-organisms. Known results range from mineral solubilization, nutrient supply and / or plant phytohormones synthesis, to biological pathogen control (Babalola, 2010).

Some positive responses in the interaction between the use of *Azospirillum brasilense* inoculation in peat medium in two rice cultivars were observed, mainly for nitrogen (N) accumulation in plants and grains were verified by Guimarães et al. (2007). These authors emphasize that inoculation associated with balanced fertilization contribute significantly to the development and production of rice grains.

Considering the root system of the rice plant, Beutler et al. (2016) report that the interaction between nitrogen fertilization and bacterial inoculation provided greater development of root and shoot system.

Similarly Vogel et al. (2013), evaluating the agronomic performance of *A. brasilense* in rice, found that the use of *A. brasilense* associated with rice culture presents promising results regarding the plant morphological aspect and the increased grain yield and a sustainable means of production.

Other excellent results were described by Moura (2011) when they found that different water slides associated with inoculation of *A. brasilense* with Ab-v5 and Abv6 strains at a concentration of 200 mL, with different N rates offered in rice cover (0, 25, 50, 75 and 100 kg ha^{-1}), increased the number of tillers by approximately 11% compared to the treatment without inoculant.

Already according to Sabino et al. (2012), inoculation using strains of diazostrophic bacteria, associated with 50 kg ha^{-1} of nitrogen, provided the largest biomass accumulations in rice seedlings.However, there are few scientific investigations that have shown concrete scientific response on the agronomic performance of *A. brasilense* in relation to inoculation of these bacteria and rice cultivation (Vogel et al., 2013).

Objective

The objective of this study was to evaluate the agronomic efficiency under field conditions of *Azospirillum brasilense* inoculant (strains Ab-V5 and Ab-V6) in the liquid and peat formulation for rice crop.

MATERIALS AND METHODS

Experimental site

The study was conducted in four different locations: Toledo, Palotina, São Miguel do Iguaçu and Cascavel all in the state of Parana (Table 1). The tests were performed from October 2016 to February 2017. The geographical coordinates, altitude and soil type of the areas are also presented, according to EMBRAPA (2013).

Determination of soil and weather conditions while conducting the experimente

The chemical and physical characterizations of the soils of the experimental areas were carried out through material analysis from samples composed by ten subsamples in each experimental area; at a depth of 0 to 0.20 m. Results are presented in Table 2.

According to the Köppen and Geiger classification (1928), the São Miguel do Iguaçu region has a subtropical humid mesothermal regional climate with hot summers (average temperature of 20.0°C and 1755 mm of rainfall for the respective regions). As for the regions of Toledo, Palotina and Cascavel, they have hot and temperate climate with hot summers (average temperature of 19.4; 20.8 and 18.2°C temperature and 1483; 1508 and 1822 mm of rainfall for the regions). Regardless of the region, there is a tendency to concentrate rainfall and winters with infrequent frost. The meteorological data of the four experimental areas during the conduction of the experiments are presented in Figures 1A, B, C and D.

Regarding the correction of these soils, when necessary, it was performed 30 days before sowing; the elevation of the base saturation was performed according to Barbosa Filho et al., 1999. The basic fertilization was performed by nutrient distribution in

Table 1. Geographic site, type of soil and climate of the places where the experiments were carried out.

Coordinates	Level	Soil type	Weather
24°40’12”S 53°39’19’W	531	Dystrophic red latosol	Humid subtropical mesothermic
24°17’44”S 53°49’40”W	267	Eutrophic red latosol	Humid subtropical mesothermic
25°23’07”S 54°12’33’W	297	Eutrophic red nitosol	Humid subtropical mesothermic
25°01’57”S 53°28’55”W	721	Eutrophic red latosol	Humid subtropical mesothermic

Table 2. Chemical and particle size characteristics of the soil collected in the 0.0-0.2m layer from the Biogenesis Experimental Station, Research and Development Center, located in Toledo - PR, 2016.

L	Chemical characteristics											Granulometry		
	pH	V	P	MO	Ca^{2+}	Mg^{2+}	K^{+}	Al^{3+}	H+Al	SB	CTC	Clay	Silt	Sand
	$CaCl_2$			-%	mg dm^{-3}			g dm^{-3}		$cmol_c$ dm^{-3}			g kg^{-1}	
1	5.42	60.19	13.28	23.09	5.77	2.19	0.38	0.00	4.32	8.22	13.21	530	378	92
2	5.21	60.15	13.92	22.28	5.09	2.27	0.75	0.02	5.09	8,49	12.29	633	305	52
3	5.78	57.25	15.22	24.02	5.05	2.05	0.99	0.00	4.97	7.96	13.03	560	358	82
4	5.86	62.15	13.28	23.17	5.56	1.28	0.69	0.00	4.91	6.41	12.99	584	371	45

P, K, Micronutrients, Mehlich^{-1} Extractor; Al, Ca, Mg, 1 mol L^{-1} KCl Extractor; H + Al pH SMP (7.5); (pH) 0.01 mol L^{-1} $CaCl_2$ extractor. Analysis performed at the Agricultural and Environmental Chemistry Laboratory, Unioeste. *Campus* Marechal Cândido Rondon, PR.

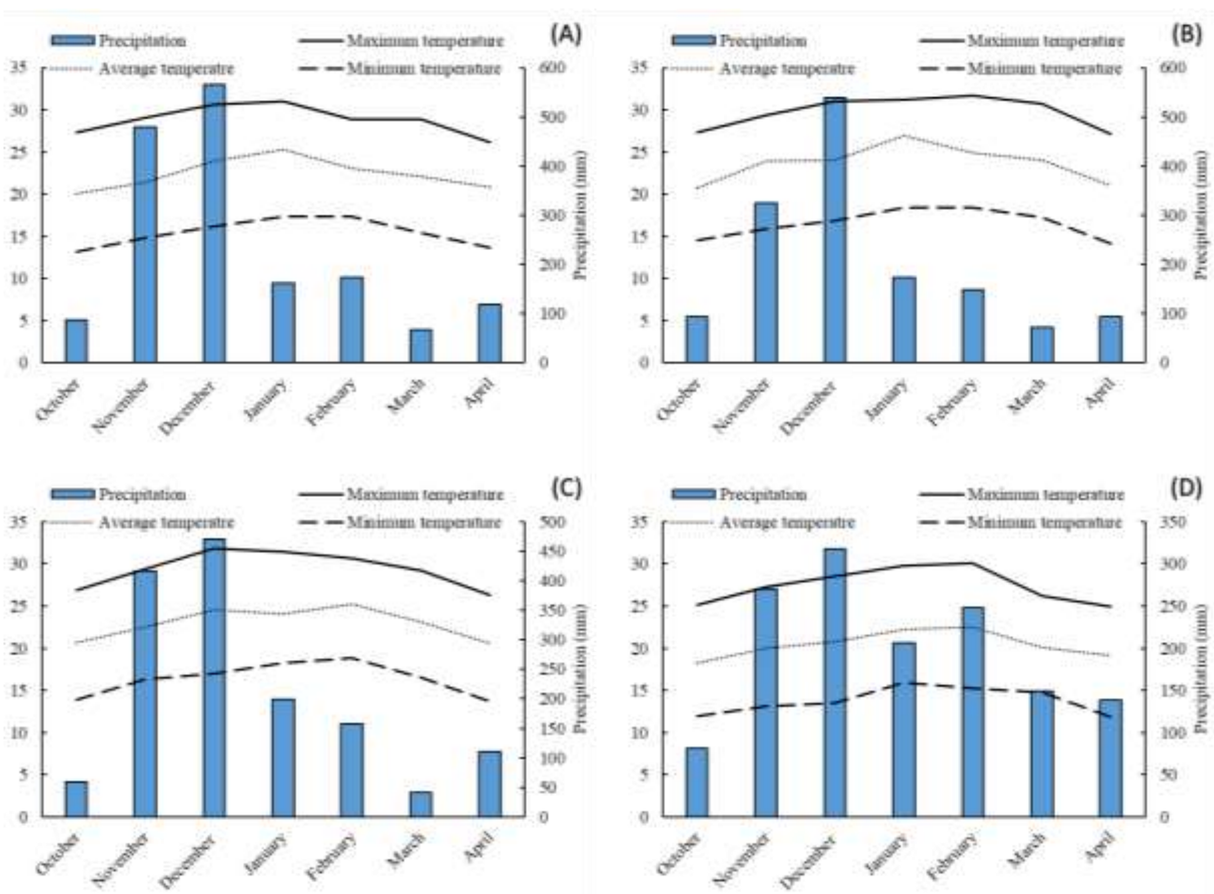

Figure 1. Average rainfall, minimum temperature (♦), average temperature (■) and maximum temperature (▲), for the different locations: A) Toledo - PR; B) Palotine - PR; C) São Miguel do Iguaçu - PR and D) Cascavel - PR, from October 2016 to February 2017.

furrows parallel to a depth of approximately 5 cm, simulating mechanical sowing.

Most likely number (MLN) of endophytic diazotrophic bacteria present in soils of experimental areas

The counting of diazotrophic microorganisms to determine the bacterial population in cell number per mL was performed by estimating the Most Probable Number (MPN) using the MacCrady table in semi-solid medium NFB, according to methodology described by Döbereiner et al. (1995).

The results of the counting of diazotrophic microorganisms in the experimental soils of the four experimental areas at the time of sowing presented population of: 1.6x 10^6 (Toledo - PR); 1.4x10^6 (Palotina - PR); 0.9x10^6 (São Miguel do Iguaçu - PR) and 1.1x10^7 (Cascavel - PR), colony-forming unit (CFU) g^{-1} of diazotrophic bacteria.

Identification of NITRO 1000 liquid and peaty rice inoculum samples used in the four experiments

According to the company, NITRO 1000 - Biological Inoculants, inoculants have the following characteristics: - Liquid rice product: Warranty: 2.0x10^8 CFU/mL *A. brasilense*, strains Ab-V5 and Ab-V6; Physical nature: liquid; Density: 1.0 g/mL; Target crop: Rice (*Oryza sativa* L.); Tested dosage for 25 kg of seed: 100 g; Lot: 400 1003 15; Manufacture: 09/05/2015 and Validity: 03/05/2016; - Liquid rice product: Warranty: 2,0x10^8 CFU/mL *Azospirillum brasilense*, strains Ab-V5 and Ab-V6; Physical Nature: Solid; Density: 1.0 g / mL; Target crop: Rice (*Oryza sativa* L.); Tested dosage for 25 kg of seed: 100 g; Lot: 410 10003 15; Manufacture: 08/28/2015 and Validity: 02/28/2016.

Identification of inoculant sample standard

In the four experiments, two standard inoculants duly registered with the Ministry of Agriculture, Livestock and Food Supply (MALFS) were used as references: - Liquid product containing *Azospirillum brasilense*: MASTERFIX L GRAMÍNEAS® commercial inoculant. The inoculant has the following characteristics: Warranty: 2.0x10^8 CFU/mL *A. brasilense*, strains Ab-V5 and Ab-V6; Physical nature: liquid; Density: 1.0 g/mL; Target crop: Rice (*Oryza sativa* L.); Test dosage used for 50 kg of seed: 100 mL; Lot: 0515843; Manufacture: 11/22/2015; Validity: 05/2016.

Quality control of tested inoculants

Inoculants from Nitro 1000 - Biological Inoculants and Standard inoculants from MASTERFIX L GRAMÍNEAS®, both used in the four experiments conducted in the different regions, were subjected to laboratory tests of concentration (Colony Forming Units), purity and characterization. The analyses followed official methods, according to Normative Instruction N° 30 of November 12, 2010 (MAPA).

The following results were obtained for the concentration of bacterial strains present in the inoculants: Liquid Standard Inoculant - MASTERFIX L GRAMÍNEAS®: 6.91x10^8 CFU mL^{-1} (Colony Forming Units); NITRO 1000 Rice Inoculant: Liquid: 4.79x10^8 CFU mL^{-1}; and NITRO 1000 Peat Inoculant: 6.12x10^8 CFU g^{-1}.

Implementation and experimental design of the four experiments

The experiments were implemented in a randomized block design with seven treatments and four replications, totaling 28 experimental plots. The treatments used were absence, presence or association between diazotrophic bacteria and nitrogen fertilization, as described below: T1, Control (absence of nitrogen fertilization and inoculation); T2, Nitrogen fertilization, 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3, Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4, Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gram 'Liquid' at the dose of 100 mL 25 kg^{-1} seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

For seed liquid inoculation aliquots of inoculants were removed according to the recommended calculations for each treatment, using a Micronam micropipette with a capacity of up to 10 mL. For the solid inoculation of the seeds, portions of inoculants were removed and measured on a Celtac model FA 2104N analytical digital scale, accurate to 0.0001 g.

Then, 2.0 kg of rice seeds were packed in high density plastic bags, where the liquid or solid inoculant was deposited directly on the seed mass according to the treatments. They were then agitated for approximately 1 min to evenly distribute the inoculant in the seeds. The seeds were placed on the bench in the shade for 2 h and then sowed.

According to the results of the chemical analysis of the soil and crop needs, basic fertilization was performed. Considering that the areas presented clay contents above 50% to 60% and P contents above 12 mg dm^3 (Table 1) were classified as good and very good, requiring fertilization of 60 kg ha^{-1} of P_2O_5. For fertilization with K, the soils were classified as very good; values above 0.31 $cmol_c$ dm^3, thus requiring the addition of 40 kg ha^{-1} of K_2O. For the distribution of fertilizers, a precision fertilizer seeder was used, equipped with an endless screw system, where at that moment the experimental area was furrowed.

Part of the urea nitrogen (100 kg ha^{-1} N) was manually applied to the sowing furrow for treatment with nitrogen fertilization. The remaining nitrogen, for the treatment that received nitrogen, was applied as a cover, at stage V3, as urea (100 kg ha^{-1} of N). The upland rice cultivar used in the four trials was IAC 201, with an average early cycle of 110 to 120 days.

Each experimental plot consisted of 10 sowing lines per 8 m in length, with 1 m spacing between plots. 0.5 m row spacing was used, with 170 plants m^{-1}, according to the cultivar specification, making an average population density of 40 kg of ha^{-1} plants. The useful experimental plot, considered for evaluations, consisted of the 6 central lines, disregarding 1 m at each end, resulting in a usable area of 48 m^2.

Prior to sowing, the seeds were treated using the fungicide Metalaxil - M + Fludioxonil (commercial product Maxim XL®) at a dose of 200 mL p.c. for 100 kg of seeds, still for disease prevention in the early stages of development, the insecticide Fipronil (commercial product Cinelli 250 FS ®) was also used, at a dosage of 150 mL of p. c. for 100 kg of seeds.

After germination, the plants were monitored for adequate development until ITreaching the S_1 stage, requiring herbicide application at the S_3 stage of the culture, with the application of Ricer (Penoxsulam) at a dose of 100 mL p.c. to 200 L ha^{-1}. The first application of fungicide treatments was performed at stage V_3 with ciproconazole (0.2 mL L^{-1}) + Picoxistrobin (0.2 mL L^{-1}) to 200 L ha^{-1}. When the plants reached the R_4 stage, the same fungicides were used, and the presence of panicle required insecticide application for insect control through the insecticide Alterne (Tebuconazole 0.75 L ha^{-1}) to 40 L ha^{-1}. One last fungicide application was carried out at the R_5 stage, with ciproconazole (0.2 mL L^{-1}) + picoxistrobin (0.2 mL L^{-1}), for 200 L ha^{-1}, at which time the insecticide Alterne was applied (Tebuconazole 0.75 L ha^{-1}) to 40 L ha^{-1}.

Evaluation of morphobiometric variables of rice plants

At the phenological stage, R_7, plant collections were performed to perform the evaluation of plant morphobiometric variables. For this, 10 plants with intact roots were collected in the second line of each experimental plot.

The plants were placed in plastic bags and immediately taken to the laboratory to measure the following variables: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen content (leaf N); and nitrogen content in grains (N grains). Plant height and stem diameter were measured with a graduated ruler and digital caliper, respectively. The dry mass variables of the different organs were obtained by weighing in a precision scale, after drying the vegetable materials in a forced air circulation oven at 65ºC, until it reached constant mass. To obtain the root dry mass and number and dry mass of nodules, the roots were washed in running water with the aid of sieves for soil removal and recovery of nodules for counting and determination of dry matter.

To determine the nitrogen content of the flag leaves, the leaves of the upper third of the plants of each useful plot were collected. The collected leaves were washed in running and distilled water and then placed in paper bags for drying in a forced air oven at 65°C for 72 h. After drying, the samples were ground and subjected to sulfuric digestion and afterwards distillation by vapor drag according to Tedesco et al. (1995) was done, then the leaf nitrogen content was determined. At the R8 phenological stage, when the plants were with the mature grains, useful plots of each treatment were harvested for subsequent determination of yield. To calculate the yield, the grain mass of the plot was corrected to 13% moisture in the humid base and the extrapolated values to kg ha^{-1}. The determination of the N content in the grains was performed with samples of the harvested grains of each plot, using the methodology already described for the determination of the N content in the leaves.

Statistical analysis

After being tabulated, the data were subjected to analysis of variance by the F test ($P \leq 0.05$) and the treatment means were compared by the Tukey test ($P \leq 0.05$). The statistical software SISVAR, version 5.3 (Ferreira, 2011) was used.

RESULTS

Experimental area in the city of Toledo/PR

By observing the results of the data obtained for the municipality of Toledo - PR, it is possible to verify a significant effect of the treatments employed for all variables tested according to Table 2 ($p \leq 0.05$) (Figure 1). In general, it can be seen in Table 3, that for all evaluated variables, superiority of treatment was obtained 40 kg ha^{-1} of N + inoculation with liquid inoculant based on *A. brasilense* at a dose of 100 mL of inoculant to 25 kg of rice seeds (T5). However, it is also possible to verify that the same treatment did not differ from 40 kg ha^{-1} N + inoculation with *A. brasilense* liquid inoculant at a dose of 150 mL inoculant for 25 kg of rice seeds for all variables tested (T6) (Table 3).

On the other hand, the lowest averages determined in this experiment occurred for all variables in the control treatment (T1), which was devoid of inoculation with *A. brasilense* and nitrogen fertilization (Table 3).

It was possible to verify, according to the tested conditions, that the inoculation of rice seeds with *A. brasilense* bacteria through the T5 liquid vehicle presented the greatest increase in the root and shoot system of 15.8 and 8.8%, respectively compare with the treatment that received the full nitrogen dose, that is 80 kg N ha^{-1} T3 (Table 3). These observed conditions reflected a gain of at least 4% in tillering and 4.6% in plant height, when also compared to the T3 treatment. On the other hand, seed inoculation with the solid vehicle (T7) also promoted gains for rice plants, exceeding by 6.4, 1.6, 2.5 and 2.3% the treatment with 80 kg N ha^{-1} (T3), in root, shoot, tiller number and plant height, respectively.

When observing the mass of 100 grains of rice for the Toledo/PR experiment, it is noted that only 40 kg ha^{-1} treatment of N + inoculation with liquid inoculant at a dose of 100 mL for 25 kg of rice seeds (T5) differed from the control treatment by 16.5%, and the others presented similar means. Similar behavior occurred for the variable N content in grains, where again the treatment with only T5 surpassed the control treatment (T1) by 18.8% and the treatment with 40 kg ha^{-1} N (T2) by 17.6%.

Likewise, the leaf N content was higher in T5 treatment, without differentiating from 40 kg ha^{-1} N + inoculation with liquid inoculant at 150 mL dose to 25 kg of rice seeds (T6). When the N content in the grains was observed, differences were observed only between 40 kg ha^{-1} of liquid inoculant N + at a dose of 100 mL to 25 kg of rice seeds, when compared to the control and applied only 40 kg ha^{-1} from N.

When evaluating the final yield, it was observed that the T5 treatment, 40 kg ha^{-1} of N + liquid inoculant 100 mL for 25 kg of rice seeds promoted the highest average, but without differentiating from 40 kg ha^{-1} of rice seeds N + liquid inoculant 150 mL for 25 kg of rice seeds, 40 kg ha^{-1} of N + Standard liquid inoculant 100 mL for 25 kg of rice seeds and 80 kg ha-1 of N ha^{-1} (Figure 2). The yield gain provided by T5 represented 13.96% compared to fertilization of 40 kg ha^{-1} N.

Experimental area in the city of Palotina/PR

When the experiment was conducted in Palotina - PR, the response patterns were conserved, with a significant effect of the treatments used for all variables ($p \leq 0.05$), and the T5 treatment promoted the highest average (Table 4). For RDM was observed superiority of 10.4% compared to fertilization with 80 kg ha^{-1} of N.

Table 3. Average biometric characteristics: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of Toledo / PR.

Treatments	RDM	SDM	TN	PH	W100	N leaf	N grains
	g plant^{-1}			Cm	g	g kg^{-1}	
T1	24.33c	171.67^{c}	72.44^{c}	97.30^{c}	2.42^{b}	29.59^{d}	6.91^{b}
T2	27.15b	168.35^{c}	76.67^{bc}	99.16^{bc}	2.56^{ab}	30.46^{cd}	6.98^{b}
T3	27.45b	179.20^{bc}	79.71^{ab}	102.35^{abc}	2.50^{ab}	30.76^{bcd}	7.45^{ab}
T4	28.62b	181.82^{abc}	81.18^{ab}	103.57^{ab}	2.62^{ab}	31.57^{bc}	7.50^{ab}
T5	31.80a	194.95^{a}	84.44^{a}	108.32^{a}	2.82^{a}	33.55^{a}	8.21^{a}
T6	29.46ab	189.00^{ab}	82.17^{a}	105.20^{ab}	2.72^{ab}	32.50^{ab}	7.67^{ab}
T7	29.22b	182.17^{abc}	81.70^{ab}	104.72^{ab}	2.60^{ab}	31.64^{bc}	7.60^{ab}
CV(%)	13.74	13.59	14.72	12.60	15.65	12.50	17.06
SMD	2.47	15.20	5.06	6.26	0.34	1.83	1.23
F cal	19.27**	8.04**	13.69**	7.79**	3.50**	11.37**	2.95**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas® 'liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Grass Peat inoculant at a dose of 100 g 25 kg^{-1} seeds.

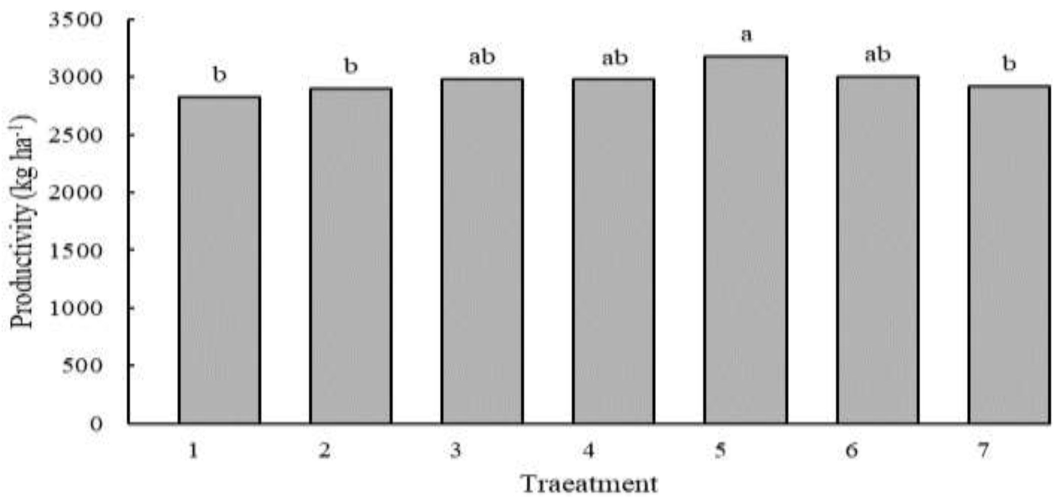

Figure 2. Productivity of cultivar IAC 201 rice plants cultivated on different forms of seed inoculation with diazotrophic bacteria, in the municipality of Toledo / PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 15.07%; SDM = 243.49; and F cal = 4.73. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Grass Peat inoculant at a dose of 100 g 25 kg $^{-1}$ seeds.

Table 4. Average biometric characteristics: root dry mass (RDM); shoot dry mass (SDM); tillering number (TN); plant height (PH); 100 grain weight (W100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of Palotina – PR.

Treatments	RDM	SDM	TN	PH	W100	Nleaf	Ngrains
	gplant^{-1}			Cm	g	gkg^{-1}	
T1	23.40^{d}	167.82^{b}	71.26^{d}	95.60^{b}	2.42^{d}	29.58^{d}	6.78^{b}
T2	26.52^{c}	167.82^{b}	76.10^{cd}	100.38^{ab}	2.53^{bcd}	30.52^{cd}	7.29^{ab}
T3	27.06^{bc}	181.30^{a}	78.59b^{c}	101.68^{ab}	2.44^{cd}	30.60^{cd}	7.24^{ab}
T4	28.86^{bc}	181.65^{a}	81.70^{ab}	101.45^{ab}	2.68^{abc}	31.55^{bc}	7.72^{ab}
T5	31.86^{a}	189.17^{a}	83.92^{a}	105.71^{a}	2.88^{a}	33.47^{a}	8.17^{a}
T6	29.55^{cb}	190.05^{a}	82.37^{ab}	103.05^{a}	2.74^{ab}	32.55^{ab}	7.80^{a}
T7	28.83^{bc}	180.07^{a}	82.35^{ab}	102.29^{a}	2.62^{abcd}	32.01^{bcd}	7.55^{ab}
CV(%)	14.47	9.84	15.83	17.66	14.26	12.51	15.65
SMD	2.92	11.91	5.24	6.30	0.26	1.84	0.98
Fcal	18.29**	12.45**	19.96**	5.20**	8.94**	11.44**	4.56**

** significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha $^{-1}$ N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg $^{-1}$ seeds.

However, for SDM, TN, PH and W100, this effect was not observed and similar averages were found between the two treatments. For these variables, it is noteworthy that treatments T6 and T4 produced similar or higher averages for some variables when compared to treatment, with 80 kg N ha^{-1} (T3) or 40 kg N ha^{-1} (T2) (Table 4).

The quantified contents of N leaf in rice plants cultivar IAC 201 were higher when using T5, without differentiating from treatment T6, these treatments had superiority, respectively 6% and 3.1% compared to the treatment that received complete fertilization 80 kg N ha^{-1}. In turn, the N content quantified in the cultivar IAC 201 rice grains was similar for practically all treatments, except for T5 and T6 that exceeded the T1 control treatment (Table 4).

The final rice yield obtained in the city of Palotina/PR showed that the treatment 40 kg ha^{-1} of N + peat inoculant promoted the highest average, but without differentiating from 40 kg ha^{-1} of N + liquid inoculant and 40 kg ha^{-1} N + standard inoculant (Figure 3). The yield gain provided by 40 kg ha^{-1} N + peat inoculant represented 10.97% and 11.46% compared to fertilization of 40 kg ha^{-1} N and 80 kg ha^{-1} N, respectively.

Experimental area in the city of Cascavel/PR

For the experiment conducted in Cascavel/PR, the response patterns observed were similar for rice cultivated in Toledo/PR and Palotina/PR. These response patterns were maintained in order to observe a significant effect of the treatments used for all studied variables ($p \leq 0.05$), and for all variables the T5 treatment promoted the highest mean, except for the SDM, which was the same followed by T6 treatment (Table 5).

For the tiller number (TN) variable in the same table, it was noted that the statistical differences observed for the treatments were smaller. The control treatments T1 and T2 presented the lowest values for this variable compared to the other treatments tested (Table 5).

The RDM variable obtained by rice plants cultivar IAC 201 was higher in inoculant treatments associated with mineral fertilization of 40 kg N ha^{-1} at a dose of 100 mL for 25 kg of rice seeds (Table 5). It is also noted in the same table, that through treatments T4, T6 and T7, it was possible to determine higher values of 14.6%; 5.4 and 8.9% against fertilization with 80 kg ha^{-1} of N (T3). This response tendency with higher averages in plants inoculated and fertilized with N were obtained in SDM, TN, W100 and N leaf (Table 5).

For the variable height of rice plants (PH), the use of 100 mL liquid inoculant for 25 kg of rice seeds associated with fertilization of plants with 40 kg N ha^{-1} produced higher plants with values higher than 5, 6% compared to80 kg N ha^{-1}; in turn the N content in the grains was similar among all treatments, with mineral nitrogen and/

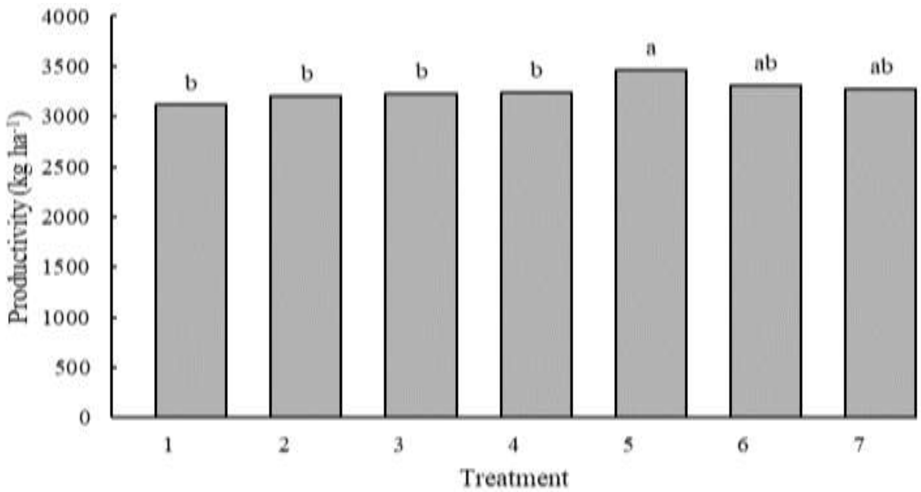

Figure 3. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria, in Palotina/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 15.07%; SMD = 243.49; and F cal = 4.73. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

Table 5. Average biometric characteristics: dry root mass (DRM); shoot dry mass (SDM); macollage number (MN); plant height (PH); 100 grain weight (W100); leaf nitrogen content (N leaf) and grain nitrogen content (N grains) of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculations with the *Azospirillum brasilense* Ab-V5 and Ab-V6 strain and fertilization nitrogenous. Municipality of Cascavel/PR.

Treatments	DRM	SDM	MN	PH	W100	Nleaf	Ngrains
	$g plant^{-1}$			cm	g	$g kg^{-1}$	
T1	24.06^{d}	173.25^{b}	73.73^{b}	102.02^{e}	2.69^{a}	30.82^{d}	7.27^{b}
T2	26.19^{cd}	173.42^{b}	77.57^{b}	103.80^{de}	2.73^{ab}	32.21^{cd}	7.92^{ab}
T3	26.43^{cd}	184.10^{ab}	82.12^{a}	104.70^{cd}	2.74^{ab}	32.04^{cd}	8.42^{a}
T4	28.98^{bc}	182.52^{ab}	84.64^{a}	105.42^{cd}	2.84^{ab}	33.01^{bc}	7.70^{ab}
T5	33.21^{a}	192.32^{a}	84.47^{a}	111.32^{a}	2.99^{a}	34.97^{a}	8.38^{a}
T6	31.50^{ab}	186.02^{a}	84.37^{a}	108.27^{b}	2.93^{ab}	33.65^{ab}	8.09^{ab}
T7	30.51^{ab}	182.87^{ab}	84.70^{a}	106.75^{bc}	2.89^{ab}	34.08^{ab}	8.05^{ab}
CV(%)	14.27	12.77	12.37	20.84	14.15	11.76	15.20
SMD	2.86	11.76	4.53	2.07	0.27	1.35	0.96
Fcal	28.61**	7.31**	21.23**	47.85**	3.75**	23.40**	3.68**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

Table 6. Average biometric characteristics: root dry mass (MSR); shoot dry mass (MSPA); tillering number (NP); plant height (ALT); 100 grain weight (M100); leaf nitrogen (N leaf) and grain nitrogen (N grain) content of 10 IAC 201 rice plants cultivated under different nitrogen fertilization concentrations and different seed inoculation with *Azospirillum brasilense* strain Ab-V5 and Ab-V6 and nitrogen fertilization. Municipality of São Miguel do Iguaçu / PR.

Treatments	RDM	SDM	NP	PH	w100	Nleaf	Ngrains
	$gplant^{-1}$			cm	g	gkg^{-1}	
T1	24.15^{e}	167.47^{c}	71.62^{d}	101.43^{c}	2.56^{c}	29.98^{d}	7.22^{b}
T2	26.22^{de}	168.17^{bc}	76.36^{c}	101.11^{c}	2.63^{c}	30.94^{cd}	7.88^{ab}
T3	27.33^{cd}	183.40^{ab}	81.22^{b}	102.54^{bc}	2.64^{bc}	31.69^{bcd}	8.77^{ab}
T4	29.22^{bc}	181.65^{abc}	82.30^{ab}	102.71^{bc}	2.81^{ab}	32.75^{abc}	7.99^{ab}
T5	32.61^{a}	189.00^{a}	83.98^{a}	106.59^{a}	2.94^{a}	34.10^{a}	8.53^{a}
T6	30.75^{ab}	184.87^{a}	83.19^{ab}	104.46^{ab}	2.89^{a}	33.44^{ab}	8.14^{ab}
T7	29.37^{bc}	180.77^{abc}	83.18^{ab}	102.81^{bc}	2.87^{a}	33.00^{ab}	8.05^{ab}
CV(%)	14.11	9.65	11.12	11.11	15.76	12.61	9.35
SMD	2.73	15.29	2.09	2.66	0.17	1.96	0.99
Fcal	23.67**	6.45**	104.09**	10.95**	15.24**	10.09**	3.39**

**Significant by the F test at 5% probability. Means followed by the same lower case letters in the column do not differ from each other by the Tukey test at 5% probability. Coefficient of variation (CV (%)); significant minimum difference (SMD). T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

or inoculation, being observed lower average in the control treatment against peat and liquid addition inoculants, 40 kg N ha^{-1}.

The ultimate productivity, again demonstrated that T5 treatment promoted the highest mean, but without differentiating from T6 and T7 treatment (Figure 4). The yield gain provided by the treatment that received 40 kg ha^{-1} of N + liquid inoculant with 100 mL for 25 kg of rice seeds, represented 6 and 8% compared to fertilization of 80 kg ha^{-1} of N and 40 kg. ha^{-1} of N, respectively.

Experimental area in the city of São Miguel do Iguaçu/PR

Finally, in the experiment conducted in São Miguel do Iguaçu/PR, significant effects were obtained from the treatments used for all variables (p≤0.05), and in all variables the T5 treatment promoted the highest average, except for SDM, where this treatment was followed by treatment with 40 kg N ha^{-1} + liquid inoculant and 150 mL for 25 kg rice seeds (Table 6).

For the city of São Miguel do Iguaçu/PR, the RDM variable of rice plants presented higher values for T5 treatment, surpassing by 11.6% fertilization with 80 kg ha^{-1} of N. The same response tendency was observed with higher averages in plants inoculated and fertilized with N for the variables SDM, MN, W100 and N leaf (Table 6).

For the variable height of rice plants (PH) the use of 100 mL liquid inoculant for 25 kg of rice seeds associated with fertilization of plants with 40 kg N ha^{-1} (T5) produced higher plants, higher than by 3.7% compared to 80 kg N ha^{-1}. In turn, the N content obtained in rice grains presented similar values among all treatments, with mineral nitrogen and / or inoculation, being observed only lower average for the control treatment compared to T5 treatment.

When observing the productivity in the municipality of São Miguel do Iguaçu/PR, they maintained the standards of Toledo/PR, Palotina/PR and Cascavel/PR, confirming the stability of the treatments employed and the agronomic efficiency of the inoculants *A. brasilense* with greater emphasis on what it used as a liquid vehicle at a dose of 100 mL for 25 kg of rice seeds (T5).

The T5 treatment promoted the highest average, however without differentiating from the T4 and T7 treatments (Figure 5). The yield gain provided by T5 treatment represented 8.9 and 4.9% compared to 40 kg ha^{-1} N and 80 kg ha^{-1} N fertilization, respectively.

DISCUSSION

The bacteria of the genus *Azospirillum* interact with rice plants naturally. In this sense, it is shown in studies developed in the state of Santa Catarina that the association between the rice plant and the *Azospirillum* bacteria occur naturally, both in roots and stalks, and

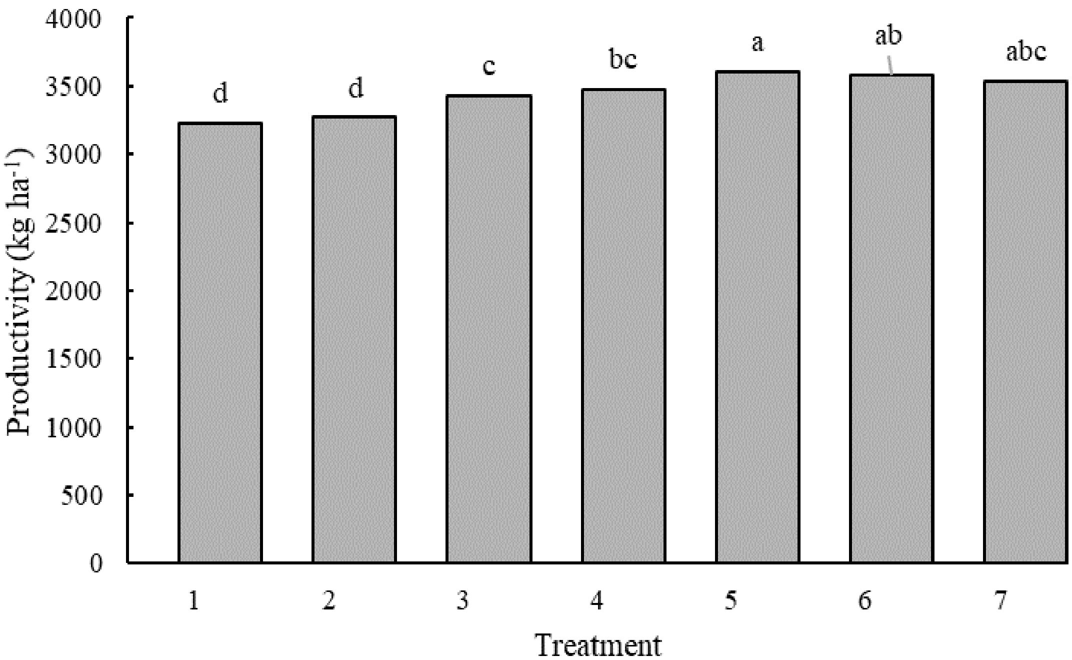

Figure 4. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria in the municipality of Cascavel/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 20.97%; SMD = 109.84; and F cal = 39.49. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg^{-1} seeds.

62.9% of the bacteria associated with roots belonging to species *A. Amazonian* (Cardoso et al., 2010). Similarly, isolates of the genus *Azospirillum* were found associated with rice cultivation (Perin et al., 2003).

In this sense, the use of this technology brings benefits to the sustainable system of agricultural production. In their study on the agronomic performance of *A. brasilense* in rice (Vogel et al., 2013), they concluded that the use of *A. brasilense* associated with rice cultivation presents promising results in relation to the sustainable production environment. This highlights a contribution in relation to the morphological aspect of the plant and increased grain yield.

The greater root and shoot development obtained in plants inoculated with *A. brasilense* is explained by the growth promoting capacity of the bacteria. It interacts with plant release growth promoting factors such as plant hormones (Perrig et al., 2007), which act as growth promoters by stimulating root and shoot development. In this sense, increments of both variables are shown when rice cultivars were studied (Guimarães and Baldani, 2013). In a study of the interaction between nitrogen fertilization and bacterial inoculation, a greater development of rice plants in both shoots and roots was observed (Beutler et al., 2016).

The increase in the number of tillers reflects the greater development of the plant in relation to the root system and shoots, since plants with higher root development have a higher capacity for water and nutrient absorption, especially N, allowing the plant to emit a larger number of plants. Tillers, in agreement with these results, showed that the inoculation of *A. brasilense* promoted gains in tiller number of 11%, which resulted in higher number of panicles (data not evaluated in this study) (Moura, 2011).

For plant height the results differ from the literature whereas Kuss et al. (2007) report that the use of *A. brasilense* does not present significant results in rice plant height; Goes (2012) found better results in plant height similar to that found in the present study. It is believed that part of this lack of results is linked to the genotypes used and the cultivation conditions, because the plant height varies according to each cultivation

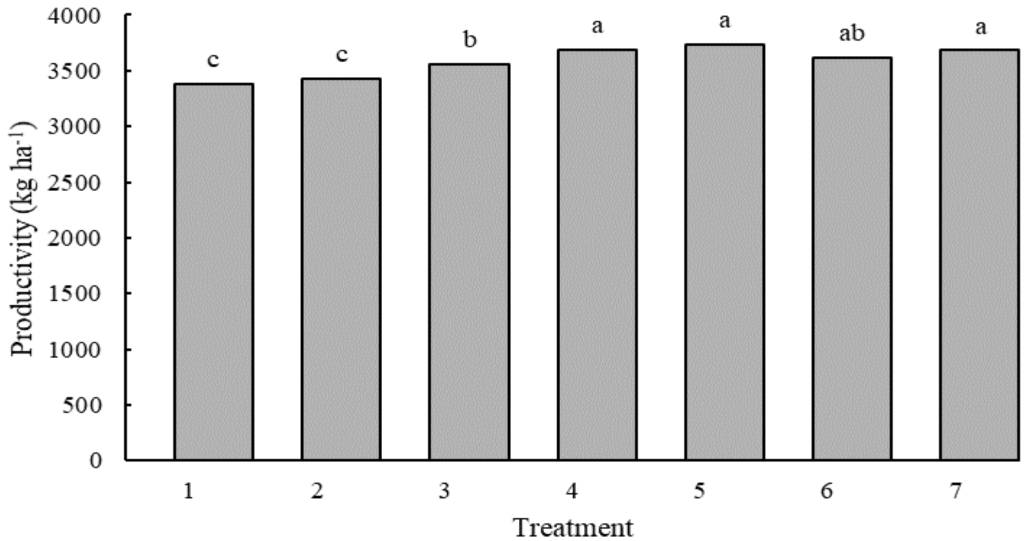

Figure 5. Productivity of rice cultivar IAC 201 cultivated on different forms of seed inoculation with diazotrophic bacteria, in São Miguel do Iguaçu/PR. Means followed by the same lower case letters in the column do not differ from each other by the 5% Tukey test; CV = 19.72%; SMD = 103.81; and F cal = 37.39. T1 - Control (absence of nitrogen fertilization and inoculation); T2 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage), and no inoculation; T3 - Nitrogen fertilization - 80 kg ha $^{-1}$ N (50% available at sowing and 50% N at flowering stage), and no inoculation; T4 - Nitrogen fertilization - 40 kg ha^{-1} N (50% available at sowing and 50% N at flowering stage) + seed inoculation with MASTERFIX L GRAMÍNEAS® 'Liquid' inoculant at a dose of 100 mL 25 kg^{-1} of seeds; T5 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Liquid' inoculant at 100 mL dose 25 kg^{-1} of seeds; T6 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas ® 'Liquid' inoculant at the dose of 150 mL 25 kg^{-1} seeds. T7 - 40 kg ha^{-1} of N (50% available at sowing and 50% N at flowering stage) + seed inoculation with Nitro 1000 Gramíneas 'Peat' inoculant at a dose of 100 g 25 kg $^{-1}$ seeds.

region.

Under the conditions tested in these four experiments, the higher plant height was a reflection of the higher crop development in the aerial part, which due to the higher presence of tillers caused it to grow in height to seek greater light exposure, but without reducing its productivity. It increases productivity.

It should also be noted that increases in plant height are not always positive from the production point of view, as they favor plant lodging; but in the present study no plant lodging was observed during the entire conduction period. Higher height development favored the increase in productivity.

Inoculated and fertilized plants with 40 kg ha^{-1} of N promoted greater N accumulation in the leaves allowing better plant nutrition and consequently greater nutrient availability for grain filling. Similar results for M100 grains are shown in a study with rice inoculation with *A. brasilense* (Gitti et al., 2012).

Gaibor et al. (2017) demonstrated that the use of *Azospirillum* application promoted higher phosphorus accumulation in plants. However, they did not observe that this increase promoted grain mass gain, being only reported increases in productivity where the association of *Azospirillum* with 140 kg N ha^{-1} promoted productivity of 5236,5 kg ha^{-1}, surpassing 791 kg ha^{-1} and 478 kg ha^{-1}, inoculation with *Azotobacter* and *Bacillus* sp., respectively. Garcia et al. (2015) demonstrated 19% gains in upland rice crop yield when inoculated with *A. brasilense* via seeds, sowing furrow or leaf spraying.

However, divergent results of the present study are shown by Gitti et al. (2012 and Beutler et al. (2016) who did not find differences between the yield of rice inoculated or not with *A. brasilense*.

The best results observed in the liquid inoculant that always promoted higher averages, even when not differing from the others, may be associated with its greater capacity to protect bacterial cells. However, the peat inoculant deserves attention especially when compared to a study with *Bradyrhizobium japonicum* inoculant. Because it has been shown that peat inoculant can provide greater protection when treating seeds with fungicides and insecticides (Vieira Neto et al., 2008), they cite cell protection as the main effect for the results. For inoculants based on *A. brasilense* it is also shown that peat provided greater survival, and it was reported that for 110 days, the concentration of $2x10^8$ CFU per gram was maintained (Ferreira et al., 2010).

Thus, the agronomic efficiency of *A. brasilense* strains Ab-V5 and Ab-V6 based inoculants for rice cultivation is demonstrated, and the liquid carrier is a good option for rice cultivation. However, the peat inoculant also shows how option, as it resembles the standard inoculant in all characteristics and locations evaluated.

Conclusion

Inoculation of rice seeds with A. *brasilense* strains Ab-V5 and Ab-V6 based inoculant increases the yield and yield components of the rice crop. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the liquid carrier showed higher agronomic efficiency for rice cultivation. The inoculant based on *A. brasilense* strains Ab-V5 and Ab-V6 using the peat medium has good agronomic efficiency similar to the standard inoculant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Areias RGBM, Paiva DM, Souza SR, Fernandes MS (2006). Similaridade genética de variedades crioulas de arroz, em função da morfologia, marcadores APD e acúmulo de proteína nos grãos. Bragantia 65(1):19-28.

Babalola OO (2010). Beneficial bacteria of agricultural importance. Biotechnology Letters 32(1):1559-1570.

Barbosa Filho MP, Fageria NK, Silva OF (1999) Correção de deficiências de micronutrientes em arroz de terras altas. Santo Antônio de Goiás: Embrapa Arroz e Feijão. P 21. (Embrapa Arroz e Feijão. Documentos 93).

Beutler AN, Burg GM, Deak EA, Schmidt MR, Galon L (2016). Effect of nitrogen-fixing bacteria on grain yield and development of flooded irrigated rice. Revista Caatinga 29(1):11-17.

Cardoso ICM, Filho O K, Mariotto JR, Miquellutti DJ, Vicente D, Neves NA (2010). Ocorrência de bactérias endofíticas do gênero *Azospirillum* em arroz irrigado no estado de Santa Catarina. Revista de Ciências Agroveterinárias 9(2):178-186.

Companhia Nacional de Abastecimento (CONAB) (2017). Acompanhamento de safra brasileira: grãos, sétimo levantamento - Safra 2015/2016. Available at: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_04_07_10_39_11_boletim_graos_abril_2016.pdf

Dobereiner J (1990). Avanços recentes na pesquisa em fixação biológica de nitrogênio no Brasil. Estudos Avançados. São Paulo 4(8):11.

Döbereiner J, Baldani VLD, Baldani JI (1995). Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas. Brasília: Embrapa-SPI P 60.

Embrapa (2013). Empresa Brasileira de Pesquisa Agropecuária – Embrapa. Sistema Brasileiro de Classificação de Solos. 3.ed. Brasília P 353.

Ferreira DF (2011). Sisvar - Sistema de análise de variância. Ciência e Tecnologia, Lavras 35(6).

Ferreira SF, Baldani JI, Baldani VLD (2010). Seleção de inoculantes à base de turfa contendo bactérias dizotróficas em duas varidades de arroz. Acta Scientiarum Agronomy 32(1):179-185.

Gaibor JR, Navarrete EC, Arteaga CC, Vasquez GG, Velez, UM, Aragon DS (2017). Eficiencia agronómica del arroz INIAP-17 con niveles de fertilización química y biológica em el Litoral Ecuatoriano. Journal of Science and Research 2(6):10-15.

Garcia NFS, Orivaldo ARF, Portugal JR, Rodrigues M, Penteado MS (2015). Rendimento e qualidade de grãos de arroz de terras altas em função de doses e modos de inoculação com *Azospirillum brasilense*. Enciclopédia Biosfera 11(21):1653-1661.

Gitti DDC, Orivaldo ARFO, Portugal JR, Corsini DCDC, Rodrigues RAF, Kaneko FH (2012). Coberturas vegetais, doses de nitrogênio e inoculação de sementes com Azospirillum brasilense em arroz de terras altas no sistema plantio direto. Bragantia 71(4):509-517.

Goes RJ (2012). Inoculação de Sementes com *Azospirillum brasilense* e Doses de N Mineral em Arroz de Terras Altas Irrigado por Aspersão. pp 30. Relatório (Mestrado em Agronomia) - Faculdade de Engenharia, Universidade Estadual Paulista, Ilha Solteira P 151.

Guimarães SG, Baldani VLD (2013). Produção de arroz inoculado com bactérias diazotróficas marcadas com resistência induzida ao antibiótico estreptomicina. Revista de Ciências Agrárias 56(2):125-132.

Guimarães SL, Baldani JI, Baldani VLD, Jacob-Neto J (2007). Adição de molibdênio ao inoculante turfoso com bactérias diazotróficas usado em duas cultivares de arroz irrigado. Pesquisa Agropecuária Brasileira 42:393-398.

Hernandes A Buzetti S, Andreotti M, Sá ME (2010). Doses, fontes e épocas de aplicação de nitrogênio em cultivares de arroz. Ciência e Agrotecnologia 34(1):307-312.

Köppen W, Geiger R (1928). Klimate der Erde. Gotha: Verlag Justus Perthes.

Kuss AV, Kuss VV, Lovato T, Flôres ML (2007) Fixação de nitrogênio e produção de ácido indolacético in vitro por bactérias diazotróficas endofíticas. Pesquisa Agropecuária Brasileira 42(10):1459-1465.

Malavolta E, Moraes MF (2007). Fundamentos do nitrogênio e do enxofre na nutrição mineral das plantas cultivadas. In: Yamada, T; Abdalla, SRS e; Vitti, GC (Ed.). Nitrogênio e enxofre na agricultura brasileira. Piracicaba: IPNI Brasil pp 189-249.

Ministério da Agricultura, Pecuária e Abastaecimento (MAPA) (2010). Instrução Normativa nº30, de 12 de novembro de 2010 (MAPA). Diário Oficial da União Federativa do Brasil.

Moura RS (2011). Lâminas de água, inoculação de sementes com Azospirillum brasilense e doses de nitrogênio em arroz terras altas. Dissertação (Mestrado em Agronomia) - Faculdade de Engenharia, Universidade Estadual Paulista, Ilha Solteira P 59.

Neves MB, Buzetti S, ARF O, Sá ME (2004). Doses e épocas de aplicação de nitrogênio em dois cultivares de arroz com irrigação suplementar. Acta Scientiarum 26(1):429-435.

Perin L, Silva MF, Ferreira JS, Canuto EL, Medeiros AFA, Olivares FL, Reis VM (2003). Avaliação da capacidade de estabelecimento endofítico de estirpes de *Azospirillum* e *Herbaspirillum* em milho e arroz. Agronomia 37(2):47-53.

Perrig D, Boiero ML, Masciarelli AO, Penna C, Ruiz AO, Cassán FD, Luna MV (2007). Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. Applied Microbiolog and Biotechnology 75(1):1143-1150.

Rojas CAL, Bayer C, Fontoura SMV, Vieiro F (2012). Volatilização de amônia da ureia alterada por sistemas de preparo do solo e plantas de cobertura invernais no centro-sul do Paraná. Revista Brasileira de Ciência do Solo 36(1):261-270.

Sabino DCC, Ferreira JS, Guimarães SL, Baldani VLD (2012). Bactérias diazotróficas como promotoras do desenvolvimento inicial de plântulas de arroz ENCICLOPÉDIA BIOSFERA, Centro Científico Conhecer 8(15):12.

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ (1995). Análises de solo, plantas e outros materiais. 2.ed. Porto Alegre, Universidade Federal do Rio Grande do Sul, pp 174p. (Boletim Técnico, 5).

USDA (2017). World agricultural supply and demand estimates. Washington D. C.: United States Department of Agriculture, P. 12.

Vieira Neto AS, Pires FR, Menezes CCE, Menezes JFS, Silva AG, Silva GP, Assis RL (2008). Formas de aplicação de inoculante e seus efeitos sobre a nodulação da soja. Revista Brasileira de Ciências do Solo 32(1):861-870.

Vogel GF, Martinkoski L, Martins PJ, Bichel A (2013). Desempenho agronômico de Azospirillum brasilense na cultura do arroz: uma revisão. Revista em Agronegócios e Meio Ambiente 6(3):567-578.