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Evaluation of the Agronomic Efficiency of *Azospirillum brasilense* Strains Ab-V5 and Ab-V6 in Flood-Irrigated Rice

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Abstract: The rational use of nitrogen fertilization is fundamental, not only to increase recovery efficiency, but also to increase crop productivity and reduce the production costs and risks of environmental impacts. In the State of Rio Grande do Sul in Brazil, irrigated rice productivity can surpass 8 tonne·ha^{-1} as a result of the technification of the crop and favorable environmental conditions, yet there is great variability in the agronomic efficiency of chemical nitrogen fertilizers, which rarely exceed 50% of the applied dose. Biological nitrogen fixation is one of the technological alternatives for reducing the use of nitrogen in this crop. In this study, the agronomic efficiency of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 in terms of biological nitrogen fixation in flood-irrigated rice cultivars in a lowland agroecosystem was evaluated through five field experiments. *A. brasilense* combined with reduced nitrogen fertilization (reduction of 30 kg N·ha^{-1}) increased the dry mass of the aerial part of rice plants by 3.2%, and promoted an increase in N concentration in stems and leaves and in the N content exported by grains by 43% and 27.5%, respectively, in relation to the absence of N and inoculant, and promoted an average increase of 30% in rice production.

Keywords: plant growth-promoting bacteria; inoculation; *Oryza sativa*; nitrogen fixation; low carbon agriculture

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1. Introduction

Rice (*Oryza sativa* L.) is widely cultivated around the world and is primarily consumed as a basic food source in Asia, Latin America, the Caribbean, and increasingly more in Africa [1]. The global consumption of rice should increase by 1.1% YoY in the coming decade, with Asian countries responsible for 70% of the projected increase, in large part driven by population growth as opposed to an increase in per capita consumption. In the various regions, only in Africa is significant growth in per capita consumption of rice projected [1]. The forecasted global rice production in 2022 is of the order of 512.6 million tonnes (beneficiary base) [2], and it is expected that rice productivity will increase by 12% due to technological and cultivation practice improvements, particularly in middle-income countries [1].

In Brazil, rice cultivation irrigated by flooding is cultivated in 1.3 million hectares of lowlands, and produces ±10,700 million tonnes of the cereal annually [3]. Flood-irrigated rice, which is concentrated in southern Brazil, provides ±70% of national production. In the southern region, one of the challenges is to increase the profitability and quality of irrigated rice, as well as reduce the risk of environmental contamination due to the high use of chemical inputs, mainly pesticides and synthetic fertilizers. In rice-growing areas of Malaysia, nitrogen fertilizers are used extensively in rice cultivation to meet the growing demands of the crop. However, the excessive use of chemical fertilizers in recent decades has led to soil toxicity by contamination with toxic heavy metals, as well as affects on the health of rice plants [4]. Commercially available fertilizers are blended with a range of trace metals, which are introduced into the soil along with the application of fertilizers [5]. Among inorganic fertilizers, phosphate fertilizers are the major source of contaminants, as they may contain traces of Cd, Pb, As, Cr, fluorine (F), strontium (Sr), thorium (Th), uranium (U), and zinc (Zn) [6]. In the south of Brazil, where some crops of irrigated rice have been cultivated for more than 100 years, a study revealed that elements (Cd, Cr, and Pb) quantified in the soil and in the crops were within the limits allowed by Brazilian legislation, with the exception of the Cd levels in the crop waters [7].

Therefore, the use of biological fertilizers to reduce chemical fertilizers is one of the most effective steps toward sustainable agriculture [8,9].

In the State of Rio Grande do Sul (RS), about one million hectares are cultivated with irrigated rice (due to flooding) that reaches a productivity of more than 8 tonnes per hectare as a result of the technification of the crop and favorable environmental conditions. In rice farming irrigated by flooding, the application of chemical nitrogen fertilizers is essential to achieve high yields. It is noteworthy, however, that there is great variability in the results inherent to agronomic efficiency, which rarely exceed 50% of the applied dose, resulting in limits to productivity and increases the cost of production [10]. Previous reports by Fageria and Baligar [11] already highlighted that the efficiency of N recovery by the flooded rice is around 40% in lowland soil. In this situation, the rational use of nitrogen fertilization is essential to not only increase recovery efficiency, but also to increase crop productivity and reduce the production costs and risks of environmental pollution. Additionally, excessive nitrogen fertilization is usually applied to maximize yields in irrigated rice fields in China, with the N recovery efficiency being around 30 to 35%, resulting in serious diffuse agricultural pollution [12]. Agroecosystem modeling processes can be applied to assess the impacts of nitrogen management alternatives on agricultural production and the environment at municipal, state, regional, federal, continental, and global scales [13], since the optimization of agronomic application of nitrogen fertilizers depends on agroclimatic variables, as well as soil, crop, and nutrient management [14]. Thus, mitigating measures are studied to reduce nitrogen fertilization and maintain and/or increase the productivity of agricultural crops, such as the use of bio-stimulants based on *Ascophyllum nodosum* extracts [15]. In addition, the increase in the cost of these fertilizers in the world market and the gap between supply and demand constitute difficulties, which is added to the possibility of gaseous losses (N_2O) by leaching and superficial runoff. In irrigated rice, N losses due to ammonia volatilization resulting from the use of urea vary from 15 (saturated soil) to 22% (moist soil) of the applied nitrogen when the interval between application of this fertilizer and the beginning of irrigation is 10 days [16]. In rice fields with wheat straw incorporation, the application of controlled-release nitrogen fertilizer in place of the conventional one can notably increase rice grain yield and improve the efficiency of N use, but have little effect on greenhouse gases, due to its stimulation in the emission of N_2O [17]. Recently, Veçozzi et al. [18] found that the technology for increasing N use efficiency with the use of controlled-release nitrogen fertilizers, as a potential alternative to reduce N losses, was similar to the behavior of uncoated urea, and did not increase the release time of the nutrient in irrigated rice cultivation. Thus, it does not constitute a management practice to minimize N losses from the irrigated rice crop. Furthermore, the outcome of the use of controlled-release nitrogen fertilizer depends on the environmental conditions of a given region, which can result in the variation of N release characteristics and synchronization with the demand of the rice crop [19]. Zheng et al. [20] report that to avoid soil degradation caused by the abundant use of chemical fertilizers and to promote high efficiency in the use of nitrogen, the combination of biochar and controlled-release nitrogen fertilizer can be applied as an effective way to achieve high yield, high fertilization efficiency, and sustainable rice production in northeast China.

In this context, addressing the loss of N in agricultural production and proposing realistic mitigation strategies based on efforts aimed at materializing associative N fixation in non-legume plants [21], especially in cereals such as rice, corn, and wheat, are some of the approaches that should be further investigated [22]. In this sense, it is important to note that, in flooded soil, the root-soil interface is the site of nitrogen fixation, and the bacteria sustaining this activity under anaerobic conditions are heterotrophic diazotrophs, with the rhizosphere being composed of microsites where this process occurs [23].

In addition, there are other problems associated with the use of N, especially when high doses are applied in the initial phase of the crop, which include promoting excessive plant growth, causing self-shading of leaves and increasing susceptibility to fungal diseases, especially to brusone [24]. The provision of high doses of nitrogen can increase the susceptibility of rice plants to brusone. This phenomenon is called nitrogen-induced susceptibility, which causes infections by the fungus *Magnaporthe oryzae* (syn. *Pyricularia oryzae*) [25]. When doses above 60 kg ha^{-1} of nitrogen are supplied without the application of fungicides, they cause an increase in brusone severity in rice panicles, a reduction in the percentage of the whole and vitreous grains, and an increase in plastered grains and plastered area [26].

Biological nitrogen fixation (BNF) is one of the technological alternatives for reducing the use of chemical nitrogen fertilizer (CNF) in this crop and is part of the Low Carbon Agriculture Program of the Ministry of Agriculture, Livestock and Supply (MAPA). The inoculation of rice plants with plant growth-promoting rhizobacteria can significantly increase rice production, thus reducing the need for nitrogen fertilizers and contributing to sustainable rice production and to reduced environmental problems [8]. Considering that diazotrophic endophytic bacteria may bring about a potential 20–30% increase in rice production [27] and interact positively with irrigated rice genotypes in lowlands (TB) [28–30], it is necessary to define the effectiveness of nitrogen doses to complement the benefit of BNF. In addition, there are strains (*Azospirillum brasilense* Ab-V5 and Ab-V6) that benefit the accumulation of N and promote the growth of grasses, increasing the biomass of the aerial part by 16.8%, with inoculation by the spraying of seeds and leaves of *Brachiaria* (*Urochloa* spp.) [31], and the N content by 25% in the species of *Urochloa brizantha* and *Urochloa ruziziensis* [32].

In this study, the agronomic efficiency of the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense*, recommended by MAPA (Normative Instruction N° 13 of 03/24/2011) for maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.), as well as for co-inoculation with rhizobia in soybean (*Glycine max* (L.) Merr.) and common bean (*Phaseolus vulgaris* L.), on the development of rice cultivars irrigated by flooding in the lowland agroecosystem is examined. The efficiency is studied to obtain conclusive information about the possibility of reducing nitrogen fertilization with the inoculation of *A. brasilense* in the soil and climate conditions of Rio Grande do Sul.

2. Materials and Methods

2.1. Edaphoclimatic Characteristics

The experiments were carried out in the field during the 2013/14, 2014/2015, 2015/16, 2016/17 and 2017/18 harvests in regions representing rice-growing areas located on the South Coast, the Internal Coastal Plain, and the Central regions of RS (Table 1), and which have different edaphoclimatic conditions. On the South Coast, where 16% of the rice in RS is sown, the experiments were carried out at the Terras Baixas Experimental Station (ETB) (Latitude: 31°52′00″ S; Longitude: 52°21′24″ S), Capão do Leão, RS, under haplic planosol soil with low clay content (Table 2). The local climate is classified as sub-tropical (Cfa—Köppen) [33], with an average annual precipitation and temperature of 1367 mm and 17.8 °C, respectively [34]. In addition to the official experiments for registration in MAPA, tests were carried out in areas of validation in commercial crops, these being rice farms in RS.

Table 1. Municipalities in the inoculant validation areas in Rio Grande do Sul. Agricultural harvests 2013/14—2017/18.

Municipalities	Agricultural Harvests				
	2013/14	2014/15	2015/16	2016/17	2017/18
Camaquã	-	-	-	-	X
Capão do Leão	X	X	-	-	-
Jaguarão	-	-	X	X	X
Mostardas	-	-	-	-	X
São Gabriel	-	-	X		-

Letter *X* indicates the agricultural season when the validation of the inoculant was carried out in the municipality and dashes indicate seasons when validations were not carried out in the municipalities.

Table 2. Soil classification, annual rainfall (PPA), latitude and longitude of the validation areas of *Azospirillum brasilense* in Rio Grande do Sul.

Municipalities	Soil Class	PPA (mm)	Latitude	Longitude
Camaquã	Melanic Gleisoil	1381	30°51′00″ S	51°48′04″
Capão do Leão	Haplic Planosol Soil	1378	31°52′00″ S	52°21′24″
Jaguarão	Haplic Gleisoil	1217	32°33′58″ S	53°22′52″
Mostardas	Haplic Gleisoil	1300	31°06′25″ S	50°55′16″
São Gabriel	Haplic Planosol Soil	1503	19°23′43″ S	54°33′59″

2.2. Research Field Experiments

2.2.1. Soil Analysis

In each crop, prior to the implementation of the experiment, soil sampling was carried out in the area, in the 0–20 cm layer, for fertility assessment, and the chemical analyses were carried out in a laboratory with a quality certificate from the Official Network of Laboratories of Soil and Plant Tissue Analysis of the States of Rio Grande do Sul and Santa Catarina, Brazil. Table 3 presents the results of chemical analysis of the soil in the experimental area of the ETB in each crop. The interpretation of the results of the chemical analysis of the soil reveals that the areas used in the five harvests had low levels of organic matter, very low levels of available phosphorus, and medium levels of extractable potassium (K) [35]. The indications for phosphate and potassium fertilization for rice were established based on the results of the chemical analysis of the soil and considering a high expectation of response of the rice crop to fertilization [36].

In each crop, prior to rice sowing, soil sampling was carried out, in the 0–10 cm layer, to estimate the natural abundance of diazotrophic microorganisms, using the most probable number method (MPN) in a medium semi-solid NFb culture [37].

Table 3. Chemical and physical attributes (0–20 cm) and population of diazotrophic bacteria (0–10 cm) in the soil of the experimental area.

Crop	pH	Al	H + Al	BS	Ca	Mg	K	Na	K	P	MO	CECe	Clay	BD
	Water (1:1)	cmol$_c$ dm^3							mg/dm^3		%		%	UFC g^{-1} Soil
2013/14	5.5	0.2	2.5	62	2.5	1.4	0.1	37	38	38	1.6	4.4	22	1.21×10^4
2014/15	5.4	0.5	2.7	49	2.3	0.2	0.1	37	31	41	1.3	3.1	18	1.58×10^4
2015/16	5.5	0.4	3.6	48	1.9	0.9	0.1	30	28	28	0.9	3.1	18	2.02×10^4
2016/17	5.5	0.0	2.7	56	2.8	1.1	0.1	-	27	37	1.2	4.3	20	1.42×10^5
2017/18	5.7	0.0	2.0	72	2.9	1.9	0.1	36	45	60	1.4	5.2	21	2.03×10^7

CECe = Effective Cation Exchange Capacity (H + Al + Ca + Mg + K); TCEC (Ca + Mg + K); BS = Base Saturation (TCEC/CEC) × 100; BD = Diazotrophic bacteria; MO = Organic matter.

2.2.2. Phytotechnical Treatments and Practices

The treatments were arranged in a randomized block design with four replications. The experimental units had dimensions of 1.58 m × 5.0 m, with nine lines and 17.5 cm line spacing that was individualized by rammed earth. The harvest area was 4.90 m^2, within each plot repeat. The sowing density was 90 kg ha^{-1} of seeds, using the cultivar BRS Pampa, with an early cycle of approximately 118 days from emergence to full grain maturation [38], with wide adaptation in the RS being implanted in the conventional cultivation system. The base fertilization consisted of the application of 300 kg ha^{-1} of formulations 00-25-25 (treatment without N and inoculation) and 05-25-25 (treatments with recommended N and without inoculation and with reduced N and inoculation) in located planting furrows.

The treatment of rice seeds (TS) was carried out in the morning of the sowing day, and, later, they were placed to dry. The sowing of inoculated rice seeds was carried out in the afternoon. The nitrogen dose used was 120 kg ha^{-1} in the coverage, established according to the results of soil analysis and recommendations for the crop, for a high expectation of a response to fertilization [36]. Nitrogen topdressing, the urea source, was applied in installments at three to four leaves (V_3/V_4) and panicle initiation (R_0) stages. In the first coverage with N, 60% of the predicted N dose was applied in the coverage treatments, and in the second, the remaining 40% was applied. The first N coverage was carried out on dry soil, immediately before the start of irrigation by flooding the soil, and the second was carried out on a non-circulating water depth. The dates of topdressing nitrogen fertilizations were estimated using the degree-day method [39]. The treatments comprised: (1) an absolute control (absence of N and *Azospirillum brasilense*); (2) a positive control with N (120 kg ha^{-1} in coverage) and, (3) a combination of nitrogen fertilization (90 kg of N ha^{-1} in coverage) and inoculation with *Azospirillum brasilense*. The control of only inoculated and without N was not included because, as previously demonstrated [40], the main benefits obtained with the inoculation of Ab-V5 and Ab-V6 strains are related to the promotion of plant growth, and not only to biological N2 fixation. In addition, in flood-irrigated rice, nitrogen is essential for the growth of this crop, is directly related to the production of dry matter [11], and is the nutrient supplied in greater quantity to increase productivity [41]. The treatments were determined based on the ANNEX to the Normative Instruction No. 13 of the Department of Agricultural Defense of MAPA, from 03/25/2011, for growth-promoting microorganisms.

2.2.3. Bacterial Strains and Inoculation

For inoculation, a commercial inoculant of the brand AzoTotal® (liquid formulation) was used, which was supplied by Total Biotecnologia Indústria e Comércio S/A (Curitiba, PR, Brazil) and used the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense*, previously selected [40] for maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) crops. This inoculant is registered in MAPA PR N° 9392310074-1 with a minimum concentration guarantee of 2.0×10^8 CFU mL^{-1}. The dose applied in the tests was 100 mL of AzoTotal® per 50 kg of seeds. 100 mL of Protege TS was also applied per 50 kg of seeds, which is an additive that guarantees better coverage of the seeds with the inoculant and optimizes the action of bacteria.

2.2.4. Variables Analyzed

At each harvest, the treatments were evaluated by determining the grain mass. This variable was measured at harvest maturation (R9 stage). For rice grain yield, a useful plot consisting of seven rows of 4 m long plants was considered. Yield data were corrected to 130 g kg^{-1} of moisture. The other variables were measured by the following determinations: N level in the rice plant, dry matter production (stems, leaves, and grains), and amount of N accumulated in the aerial part of the rice plants (stems and leaves). The level of N in the plant was evaluated by the variables relative chlorophyll index (CRI) and N content in the rice flag leaf. The CRI was measured with a SPAD 502—Minolta chlorophyll meter on the flag leaf of ten plants per experimental unit at the flowering stage (R4). The N content in the rice leaf was determined in samples consisting of the flag leaf of 40 plants sampled from each experimental unit, using the method described by Freire [42]. The nitrogen exported by the grains of samples collected from the production area (4.90 m^2) was also determined.

2.2.5. Statistical Analysis

In this work, a joint analysis of the planned experiments was carried out in randomized block designs in divided plots with four replications. The *Azospirillum* inoculant factor was allocated in the treatments, and the other factor was allocated to agricultural crops. Thus, the results obtained were initially submitted to the test of normality of variables and homogeneity of variances. Then, analysis of variance (ANOVA) with 95% confidence was tested. When the F value was significant, the means were compared using the Tukey test ($p < 0.05$). The statistical software R [43] was used.

2.3. Experiments in Validation Fields

The experiments were carried out in five municipalities with different soil and climate conditions in Brazil, in five agricultural seasons (Tables 1 and 2), using the cultivars BRS Querência, BRS Pampa, BRS Pampeira, Guri INTA CL, and IRGA 424 RI.

2.3.1. Soil Analysis

The attributes of the soils of the validation areas were determined, in each agricultural season, before the implantation of the experiments. Table 4 shows the results of chemical analysis of the soil of the experimental validation areas in each agricultural season.

Table 4. Chemical and physical attributes of the soils (0–20 cm) of the validation areas.

Municipalities	pH	Al	H + Al	BS	Ca	Mg	K	Na	K	P	M.O.	CECe	Clay
	Water (1:1)			cmol$_c$ dm^3					mg/dm^3		%		%
Camaquã	5.3	0.3	5.5	55	4.8	1.9	0.2	-	26	12	2.7	12.2	17
Capão do Leão	5.6	0.1	3.0	48	1.8	0.8	0.1	-	32	3.1	1.1	2.9	20
Jaguarão	5.5	0.2	2.5	62	2.5	1.4	0.1	37	38	3.8	1.6	4.4	22
Mostardas	4.7	0.2	3.1	37	1.0	0.6	0.3	-	88	12	1.4	4.9	13
São Gabriel	5.2	0.3	4.7	82	16.8	4.2	0.5	-	200	10	2.6	21.9	20

CECe = Effective Cation Exchange Capacity (H + Al + Ca + Mg + K); TCEC (Ca + Mg + K); BS = Base Saturation (TCEC/CEC) × 100; MO = Organic matter.

2.3.2. Phytotechnical Treatments and Practices

The treatments were installed in adjacent plots with uniform soil in a completely randomized design implanted in the minimum tillage system. The base fertilization carried out at the time of sowing consisted of 300 kg ha^{-1} of the formula 05-20-20 (N-P-K), and the nitrogen fertilization coverage was according to that used by the producer (Table 5). Nitrogen fertilization coverage was applied in installments, at the three to four leaf (V_3/V_4) and panicle initiation (R_0) stages. In the first coverage with N, 60% of the N dose predicted in the coverage treatments were applied, and, in the second, the remaining 40% was applied. The first N coverage was carried out on dry soil, immediately before the start of irrigation by flooding the soil, and the second was carried out on a non-circulating water depth. The treatments consisted of nitrogen fertilization used by the producer for rice grown in the sowing system in dry soil and a combination of 50 and 75% of nitrogen fertilization (N in coverage), as well as inoculation with *Azospirillum brasilense*, with two inoculant formulations (liquid and peaty) applied in seed treatment. Urea was the source of N used for coverage (Table 5).

Table 5. Nitrogen doses used in rice crops in each validation area as a function of the combined treatments with *Azospirillum brasilense* in the liquid and peaty formulations.

Validation Areas	Treatments *				
	1	2	3	4	5
Nitrogen Fertilizer (kg N ha^{-1})					
Camaquã	162	132.7			
Capão do Leão	90	120 *	60		
Jaguarão	120	90	90		
Mostardas	142	142			
São Gabriel	67.5	60	90	60	90
Azospirillum brasilense (mL or g 50 kg seeds^{-1})					
Camaquã		150 mL			
Capão do Leão		100 mL	100 mL*		
Jaguarão		150 mL	200 g**		
Mostardas		150 mL			
São Gabriel		150 mL	150 mL	200 g	200 g

* 120 kg ha^{-1} = Recommended dose of N [36]. *mL = liquid formulation; **g = peaty formulation.

2.3.3. Evaluated Products

The commercial inoculant brand AzoTotal® (liquid and peaty formulations), which was supplied by Total Biotecnologia Indústria e Comércio S/A (Curitiba, PR, Brazil), used the Ab-V5 and Ab-V6 strains of *Azospirillum brasilense*, with a guaranteed concentration minimum of 2.0 × 10^8 CFU mL^{-1}, in the validation areas. The doses applied were 150 mL per 50 kg of seeds (liquid formulation) and 200 g per 50 kg of seeds (peaty formulation). A total of 100 mL of the additives Protege TS® and Lastro® were also applied per 50 kg of seeds, which was supplied by Total Biotecnologia Indústria e Comércio S/A (Curitiba, PR, Brazil) in order to provide a better coating of the seeds with the inoculant and to optimize the action of bacteria.

2.3.4. Variables Analyzed

At each season, the treatments were evaluated by determining the grain mass, mass, and number of panicles. These variables were measured at the harvest maturation stage (R9). For rice grain yield, a total plot was considered useful. Yield data were corrected to 130 g kg^{-1} of moisture.

2.3.5. Statistical Analysis

The data obtained were submitted to the test of normality of variables and homogeneity of variances. Then, they were submitted to analysis of variance (ANOVA) with 95% confidence. When the F value was significant, the means were compared using the Tukey test ($p < 0.05$). The statistical software R [43] was used. For the 2013/14 data set, the statistical test utilized was the Kruskal–Wallis ($p < 0.05$), because the data did not display homogeneity of variance. For the other data set years, the requirements of the ANOVA were met, and thus the test of means was conducted according to the Tukey test.

3. Results

3.1. Field Research Experiments

The average estimate of the natural abundance of diazotrophic microorganisms in the soil was 1.21 × 10^4 CFU g^{-1} of soil in the first crop, while, after four agricultural crops, the soil was enriched with a population of 2.03 × 10^7 CFU g^{-1} of soil (Table 3). In this way, the benefits of inoculating rice seeds with *Azospirillum brasilense* for improving the diazotrophic bacterial community in the soil in rice production systems in rotation with soybean in crop and livestock integration in lowlands were proven.

In Table 6, the average values of the dry mass of the aerial part of the rice plants are presented in the five agricultural seasons. The use of the inoculant *A. brasilense* in combination with the reduced dose of nitrogen fertilization (90 kg of N ha^{-1}) had a marked effect on the production of dry matter of the aerial part of the rice plants, resulting in a greater amount of mass over the course of a period of time of five agricultural crops. It is noteworthy that the production of dry matter achieved by the use of *A. brasilense* was equivalent to that provided by the use of the recommended full dose of N for rice (120 kg of N ha^{-1}). In the absence of nitrogen fertilization and inoculant, the lower production of dry matter was similar in the seasons and in relation to the other treatments, with the exception of the 2014/15 season, where the amount of mass was higher.

Table 6. Production of dry matter of rice plants (kg ha^{-1}) as a function of treatments with and without nitrogen and combined with *Azospirillum brasilense*. Means of four applications. Crops 2013/14–2017/18.

Treatments	Agricultural Crops				
	2013/2014	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**
Control	4522 bB	8041 aA	7593 bAB	6805 bAB	4578 bB
120 N *	7081 abB	9623 aAB	10,619 aA	9498 aAB	7709 aAB
90 N + Azo **	7582 aA	9560 aA	10,434 aA	9783 aA	8599 aA
CV (%)			5.7		

Averages followed by the same letter, lowercase in the columns (compare treatments), and uppercase in the rows (compare crops), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. * 120 kg of N ha^{-1}. ** 90 kg of N ha^{-1} + *Azospirillum brasilense.*

Table 7 shows the mean values of nitrogen in the aerial part (stems + leaves) of the rice plants in the five agricultural seasons. The effect of inoculation with a reduced dose of N (90 kg ha^{-1}) was manifested with equal amounts of N accumulated in the rice plants compared to the recommended dose of N (120 kg ha^{-1}) in three seasons. The lowest N accumulation (0.12%) was observed in the absence of nitrogen fertilization and inoculant (2014/15 harvest), while the highest amount of N accumulated (1%) in rice plants was in the presence of 120 kg of N ha^{-1} (2016/17 harvest). These results show that, in general, *A. brasilense* promotes an increase in N accumulation in the stems and leaves of rice plants.

Table 7. Amount of nitrogen accumulated in the area of rice plants (%) as a function of treatments with and without nitrogen and combined with *Azospirillum brasilense*. Average of four repetitions. Crops 2013/14–2017/18.

Treatments	Agricultural Crops				
	2013/2014	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**
Control	0.57 bA	0.12 cB	0.52 aA	0.48 cA	0.63 aA
120 N *	0.88 aAB	0.72 aBC	0.52 aD	1.00 aA	0.63 aCD
90 N + Azo **	0.84 aA	0.49 bC	0.67 aABC	0.70 bAB	0.60 aBC
CV (%)			12.3		

Averages followed by the same letter, lowercase in the columns (compare treatments), and uppercase in the rows (compare crops), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. * 120 kg of N ha^{-1}. ** 90 kg of N ha^{-1} + *Azospirillum brasilense.*

Table 8 shows the average values of nitrogen exported by the grains of rice plants in the five agricultural seasons. The amount of N exported by the grains using the inoculant with the reduced dose of N (90 kg ha^{-1}) did not differ from the treatment with the recommended dose of N (120 kg ha^{-1}), in the five seasons. Thus, the benefit of *Azospirillum brasilense* to promote the growth of the plant root system has been highlighted and, consequently, to increase the absorption of N that will be translocated to the grains. The inoculant *A. brasilense*, combined with the application of 90 kg of N ha^{-1}, provided an average increase of 27.5% in the content of N exported in relation to the control with the absence of N and inoculant.

Table 8. Amount of nitrogen exported by the grains of rice plants (%) as a function of treatments with and without nitrogen and combined with *Azospirillum brasilense*. Average of four repetitions. Harvests 2013/14–2017/18.

Treatments	Agricultural Crops				
	2013/2014	**2014/2015**	**2015/2016**	**2016/2017**	**2017/2018**
Control	0.98 bA	0.64 bB	0.69 aB	0.79 bAB	0.93 aA
120 N *	1.40 aA	1.07 aB	0.82 aC	1.15 aB	0.98 aBC
90 N + Azo **	1.30 aA	1.00 aBC	0.81 aC	1.03 aB	1.00 aBC
CV (%)			16.6		

Averages followed by the same letter, lowercase in the columns (compare treatments), and uppercase in the rows (compare crops), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. * 120 kg of N ha^{-1}. ** 90 kg of N ha^{-1} + *Azospirillum brasilense*.

In Figure 1, the average values of productivity of the five agricultural harvests are presented. The data show the significant effects of treatments with the use of the inoculant *A. brasilense* on rice productivity. The treatment with 75% nitrogen fertilizer, in the topdress (90 kg of N ha^{-1}) (T3) associated with the liquid inoculant, showed similar behavior to the control with 100% N (T2) in four agricultural seasons, but differed in the agricultural season 2013/14, which showed an increase in grain yield of 19%. In the 2014/15 crop, there was no distinction between treatments with *A. brasilense* and the absence of an inoculant. In the average of five harvests, it was observed that the inoculation, combined with a reduction of 30 kg of N ha^{-1} of the topdressing fertilization, provided the same productive level as the complete mineral nitrogen fertilization (100%N). It is also noted that *A. brasilense* provided a 30% increase in irrigated rice productivity, compared to the non-inoculated control with the absence of nitrogen fertilization.

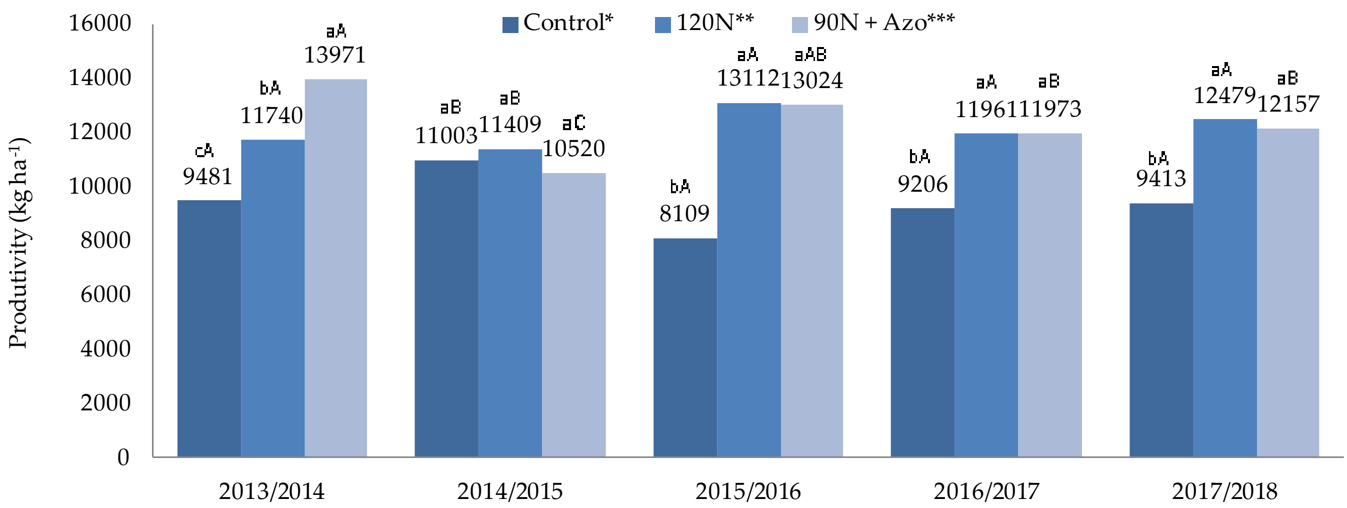

Figure 1. Mean yields of rice grains (kg ha^{-1})[1], over the 5-year period of the 2013/14 to 2017/18 agricultural seasons, as a function of inoculation with *Azospirillum brasilense* (strains Ab-V5 and Ab-V6). Data represent the means of five repeat field experiments, each with four replicates. When followed by the same letter, lowercase (compare treatments) and capital letters (compare crops) do not differ significantly from each other according to the Tukey test in $p \leq 0.05$. [1] coefficient of variation = 14.8%. * Absence of N and inoculant. ** 120 kg of N ha^{-1}. *** 90 kg of N ha^{-1} + *Azospirillum brasilense*. The shades of the vertical bars indicate the treatments: dark blue (control, noninoculated); blue (complete nitrogen fertilization); light blue (seed inoculation with *Azospirillum brasilense* and reduced nitrogen fertilization).

3.2. Experiments in Validation Fields

The evaluations of the agronomic efficiency of the inoculant with *A. brasilense* (strains Ab-V5 and Ab-V6) in irrigated rice fields under different edaphoclimatic conditions, in RS (Brazil), are in Tables 9 and 10.

In Camaquã, the final production differed between treatments (Table 9). The BRS Pampeira cultivar presented a productivity of 12,180 kg ha^{-1} (243.61 bags ha^{-1}) with the use of *A. brasilense* (T2) and reduced nitrogen fertilization, which was 3.6% higher than the production with 100% of the fertilization nitrogen (T1) which was 11,750 kg ha^{-1} (235 bags ha^{-1}). The 20% reduction in nitrogen fertilization, which corresponded to 30 kg of N ha^{-1}, provided an increase of 8.6 bags ha^{-1}, that is, R$ 312.00 ha^{-1}, based on the commercial price of rice (50 kg clean and dry) in the 2017/18 harvest.

In Capão do Leão, there was a significant interaction of nitrogen rates (50 and 75% of fertilizer N) associated with *A. brasilense* and with the cultivars BRS Pampa and BRS Querência (Table 9). The cultivar BRS Querência showed higher productivity in the 2013/14 and 2014/15 seasons, of 10,475 kg ha^{-1} and 10,256 kg ha^{-1}, respectively, with 50% of the N dose combined with the inoculant. This resulted in an average grain increment, in relation to the 100% dose of N fertilizer, of 13%. As for BRS Pampa, for the 2013/14 crop, there was no significant difference in the doses of N mineral fertilizer (50%, 75%, and 100% of the recommended dose) combined with the inoculant. However, it was observed that the application of 60 and 90 kg of N ha^{-1}, combined with *A. brasilense*, allowed an average productivity of 11,000 kg ha^{-1}. In the 2014/15 crop, there was a significant difference between treatments, and results showed higher productivity with 100% N fertilizer and no inoculation. In the same way, the dose of 75% of N fertilizer with *A. brasilense* stood out again, with an average grain yield to the order of 11,000 kg ha^{-1}.

In Jaguarão, the final production differed between treatments and between the evaluated cultivars (Table 9). In the 2015/16 crop, the cultivar BRS Querência showed the highest production with 90 kg of N ha^{-1} applied in coverage combined with the peaty inoculant (8318 kg ha^{-1}), which was 9.4% higher than the treatment with 100% of the nitrogen fertilization. In the 2016/17 crop, the BRS Pampa cultivar showed the highest production with 90 kg ha^{-1} of N applied in coverage combined with the liquid inoculant (8545 kg ha^{-1}), which was 4.6% higher than the treatment with 100% of the nitrogen fertilization. In the 2017/2018 harvest, the cultivar Guri INTA CL showed the highest production with 90 kg of N ha^{-1} applied in coverage combined with the liquid inoculant (8568 kg ha^{-1}), and had no overshoot in relation to the treatment with 100% of the nitrogen fertilization. These results demonstrate the importance of nitrogen fertilization for irrigated rice, as well as the contribution of *A. brasilense* to obtain an average productivity of 8411 and 8272 kg ha^{-1}, respectively, with the use of liquid and peaty inoculants for cultivars with different agronomic characteristics.

In Mostardas, the final production differed between treatments. The BRS Pampa cultivar presented a productivity of 10,591 kg ha^{-1} (211.8 bags ha^{-1}) with the use of liquid inoculant (T2), which was 38.6% higher than the production with 100% nitrogen fertilization (T1) at 7639 kg ha^{-1} (152.7 bags ha^{-1}). The combination of *A. brasilense* (strains Ab-V5 and Ab-V6) with nitrogen fertilization on the farm provided an increase of 59 bags ha^{-1}, i.e., R$ 2140.52 ha^{-1}, based on the commercial price of rice (50 kg clean and dry) from the 2017/18 harvest.

In São Gabriel, final production differed between treatments (Table 9). The treatment with 75% nitrogen fertilizer on top (90 kg of N ha^{-1}) combined with the peat inoculant showed the highest grain yield (9504 kg ha^{-1}), which differed from the treatments with 100% nitrogen fertilizer and 75% nitrogen fertilizer and liquid inoculant. This result also demonstrates the efficiency of *A. brasilense* in peaty formulation for rice grain yield.

Table 9. Yield of rice grains (kg ha^{-1}) as a function of inoculation with *Azospirillum brasilense* (strains Ab-V5 and Ab-V6). Averages of the 2013/14, 2014/15, 2015/16, 2016/17 and 2017/18 harvests.

Validation Areas	Treatments *					Agricultural Crops	Cultivar
	1	2	3	4	5		
Camaquã	11,750 b	12,180 a				2017/18	BRS Pampeira
CV (%)				12.8			
Capão do Leão	9823 b	9238 c	10,475 a			2013/14	BRS Querência
	11,054 n.s.	11,172 n.s.	10,946 n.s.			2013/14	BRS Pampa
	9596 b	8762 c	10,256 a			2014/15	BRS Querência
	11,129 b	12,463 a	10,375 c			2014/15	BRS Pampa
CV (%)				13.2			
Jaguarão	7600 b	8121 a	8324 a			2015/16	BRS Querência
	8166 a	8545 a	8318 a			2016/17	BRS Pampa
	9003 a	8568 b	8324 b			2017/18	Guri INTA CL
CV (%)				10.8			
Mostardas	7639 a	10,591 b				2017/18	BRS Pampa
CV (%)				11.7			
São Gabriel	6593 c	8400 ab	9055 ab	7531 bc	9504 a	2015/16	Guri INTA CL
CV (%)				12.8			

Means followed by the same letter, lowercase in the lines, do not differ significantly from each other by the Tukey test ($p < 0.05$). Coefficient of variation [CV (%)]. * Nitrogen fertilization of coverage and inoculant: Camaquã (T1) 162 kg of N ha^{-1}, (T2) 132.7 kg of N ha^{-1} + liquid inoculant; Capão do Leão (T1) 90 kg of N ha^{-1} + liquid inoculant, (T2) 120 kg of N ha^{-1}, (T3) 60 kg of N ha^{-1} + liquid inoculant; Jaguarão (T1) 120 kg of N ha^{-1}, (T2) 90 kg of N ha^{-1} + liquid inoculant; (T3) 90 kg of N ha^{-1} + peaty inoculant; Mustards (T1) 142 kg of N ha^{-1}, (T2) 142 kg of N ha^{-1} + liquid inoculant; São Gabriel (T1) 67.5 kg of N ha^{-1}, (T2) 60 kg of N ha^{-1} + liquid inoculant, (T3) 90 kg of N ha^{-1} + liquid inoculant, (T4) 60 kg of N ha^{-1} + peat inoculant, (T5) 90 kg of N ha^{-1} + peat inoculant.

Table 10. Dry mass production and the number of panicles of rice plants as a function of treatments in three validation areas Means of three replications.

Validation Areas	Cultivar	Treatments *	Dry Mass of the Aerial Part g m^{-2}	Number of Panicles m^2
Camaquã	BRS Pampeira			
		1	1307.06 a	552 a
		2	1286.61 a	553 a
CV (%)			8.80	4.4
Jaguarão	Guri INTA CL			
		1	288.05 ab	552 a
		2	332.60 a	550 a
		3	225.80 b	390 b
CV (%)			9.20	7.4
Mostardas	BRS Pampa			
		1	716.02 b	363 b
		2	1007.74 a	533 a
CV (%)			11.20	6.2

Means followed by the same letter, lowercase in the rows, do not differ significantly by Tukey's test ($p < 0.05$). Coefficient of variation [CV (%)]. * Cover nitrogen fertilization and inoculant: Camaquã (T1) 162 kg N ha^{-1}, (T2) 132.7 kg N ha^{-1} + liquid inoculant; Jaguarão (T1) 120 kg N ha^{-1}, (T2) 90 kg N ha^{-1} + liquid inoculant; (T3) 90 kg N ha^{-1} + peat inoculant; Mostardas (T1) 142 kg N ha^{-1}, (T2) 142 kg N ha^{-1} + liquid inoculant.

4. Discussion

In this work, we demonstrated that *A. brasilense* in rice, besides the agronomic potential for saving about 30% of N, involves the employment of an input of biological nature that benefits plant root growth promotion and increases resistance to biotic and abiotic stresses. Additionally, plant growth-promoting diazotrophic bacteria can improve the ability of rice plants to assimilate N from the soil, and represents a viable economic and environmental strategy to improve pasture production as well [31]. Another fact is that a high N supply to rice plants does not allow for optimal photosynthetic rates and increases the likelihood of photoinhibition due to an imbalance of sunlight absorption and utilization [44]. Another study reported that regardless of the inoculation method—in seeds or by foliar application—*A. brasilense* strains Ab-V5 and Ab-V6 promoted corn plant growth, which was a reflection of the application of cells and metabolites that promoted both the synthesis of phytohormones and the induction of plant defense-related genes [45].

We also highlight the alignment of BNF in rice with the Sustainable Development Goals (SDGs) of the United Nations Sustainable Development Summit and the United Nations (UN) Sustainable Rice Platform (SRP), with action for innovation in the relations between producers and the Brazilian government regulatory bodies. Concerns about the N economy, efficiency, and impact on the environment have renewed interest in exploring alternative or supplemental sources of N for sustainable agriculture [46]. BNF in irrigated rice constitutes an alternative source in organic production systems and a supplementary source to reduce nitrogen fertilization in chemical production systems. It also presents itself as a "bio-economy" practice to replace fossil and non-renewable resources.

In the experimental field, *A. brasilense*. (strains Ab-V5 + Ab-V6) combined with a reduced nitrogen fertilizer dose using seed inoculation with a liquid formulation promoted an average increase of 30% in rice production when compared with the absence of nitrogen fertilizer. On the other hand, in the 2014/2015 season experiment, plants with no nitrogen fertilizer and inoculant showed higher shoot dry mass production. This result was associated with climatic factors, such as temperature and solar radiation, which occurred in this season and were favorable to productivity [47]. Although, the productivity variable tends to decrease with the absence of nitrogen coverage application, which is in agreement with Scivittaro and Machado's observations [48] that nitrogen requirements are higher in the tillering and reproductive phases, and it is in this last phase that the plant presents greater efficiency in the absorption of N for grain production.

A similar result was found with the highest agronomic efficiency of inoculation of rice seeds with the liquid inoculant based on *A. brasilense* (strains Ab-V5 + Ab-V6) in experiments carried out in the municipalities of Toledo, Palotina, Cascavel, and São Miguel do Iguaçu in the state of Paraná, Brazil [49].

Field studies indicate that associative N_2 fixation can potentially contribute amounts of N (>30–40 kg N ha^{-1} yr^{-1}) to the nutrition of plants that are important in tropical agriculture, including sugarcane (*Saccharum* sp.) and forage grasses (*Panicúm maximum*, *Brachiaria* sp. And *Leptochloa fusca*), when cultivated in non-inoculated and N-deficient soils. Data from pot experiments indicate that rice can naturally benefit from associative N_2 fixation and that inoculation responses may occur due to N_2 fixation [50].

In our work, the practice of inoculating rice seeds with *Azospirillum* allowed for a reduction of 30 kg of N ha^{-1} and, consequently, contributed to reducing the emission of greenhouse gases. The agronomic efficiency (AE) in the use of N by irrigated rice was obtained by the difference between the grain yield in the nitrogen treatments (nitrogen fertilizer 120 kg of N ha^{-1}), treatments with *A. brasilense*, and nitrogen fertilizer (90 kg of N ha^{-1}), and treatments with the absence of nitrogen fertilization and inoculant, divided by the amount of N applied, according to the following formula [51]:

$$AE = (PG_{cf} - PG_{sf})/QN_a, \text{ given in } kg \cdot kg^{-1}$$

where PG_{cf} = grain yield with fertilizer, PG_{sf} = grain yield without fertilizer, and QN_a = amount of nutrient applied, all expressed in $kg \cdot ha^{-1}$.

The results of the AE of 32 kg grains kg^{-1} of N applied with *A. brasilense* and 90 kg of N ha^{-1} and of 22 kg grains kg^{-1} of N applied with 120 kg of N ha^{-1}, demonstrate an economic productivity that is 45% higher for grains with the inoculation of rice seeds with *A. brasilense*.

In our work, in five consecutive agricultural seasons, average yields above 12,000 kg ha^{-1} in the BRS Pampa cultivar were observed with the combined use of *A. brasilense* and reduced mineral nitrogen fertilization. The productivity differential of the combination of 90 kg of N ha^{-1} (covering fertilization) with the inoculation in relation to the treatment of 120 kg of N ha^{-1} was 1.5%, which corresponded to about four bags ha^{-1}. Considering that the price of paddy rice (50 kg bag) was BRL 83.82 (May 2021) [52], there was a gain of BRL 335.28 ha^{-1} with the difference in bags obtained with the use of *A. brasilense* (strains Ab-V5 + Ab-V6). Regarding the cost, with the average price of 1 kg of nitrogen fertilizer (urea source) in October 2022 in Brazil (US$0.802; CONAB; 1 US$ = R$5.36), which corresponds to US$802 per tonne of urea (US$1.78 kg of N), the farmer saving 30 kg of N ha^{-1} saves US$53.4 ha^{-1}. As the cost of inoculation is about US$2 ha^{-1}, the savings would be over US$51 ha^{-1}.

In the validation fields, *A. brasilense* combined with nitrogen fertilization with a 25% reduction in the coverage fertilization (90 kg of N ha^{-1}) and with the cover fertilization adopted by the producer showed agronomic efficiency in obtaining high productions of rice grains. The highest yield was obtained with the cultivar BRS Pampeira (12,180 kg ha^{-1}), which resulted from the use of *A. brasilense* combined with a 20% reduction in nitrogen fertilization (119.2 kg of N ha^{-1}), which resulted in an increase of 8.6 bags ha^{-1} compared to the control treatment (without the reduction of the nitrogen fertilization of cover).

In economic terms, the inoculation technology of rice seeds with *A. brasilense* (strains Ab-V5 and Ab-V6) for different cultivars increased the net profit per hectare with the adoption of the inoculant combined with nitrogen fertilization for: BRS Pampeira (R $318.08), BRS Pampa (BRL 2025.91), and IRGA 424RI (BRL 677.11) [53]. The economic viability analysis was based on the change in management (adoption of the use of inoculant instead of nitrogen fertilization adopted by the producer) using the partial budgeting technique. The price of rice considered was US$ 6.51 (1 US$ = R$ 5.36) sc^{-1} of 50 kg during the 2017/18 harvest (03/2018), and US$ 7.45 (1 US$ = R$ 5.36) sc^{-1} of 50 kg, during the 2018/19 harvest (03/2019). The prices of nitrogen fertilizer (urea) were US$ 0.150 (1 US$ = R$ 5.36) kg^{-1}, the commercial inoculant was US$ 1.86 (1 US$ = R$ 5.36) vial^{-1} of 10 mL, and the inoculant additive was US$ 1.30 (1 US$ = 5.36) ha^{-1}, in effect at the sowing time of the 2017/18 (10/2017) and 2018/19 (10/2018) harvest.

The results of these validations demonstrate that, in lowland agroecosystems, it is possible to obtain higher-than-average yields of rice irrigated by flooding in RS with the practice of inoculating seeds with an inoculant containing *A. brasilense*. Similar results with the inoculation of grain-producing grasses with *A. brasilense*, in different soil and climatic conditions in Brazil, have been previously reported for rice [49], corn and wheat [40], and wheat [54].

We also highlight the importance of BNF in irrigated rice for systems with official government certifications, such as Organic Rice Production, which needs alternatives for nitrogen supply, given the high demand of the nutrient for this cereal and the fact that the use of fertilizers obtained through industrial processing would not be allowed [55].

Thus, the results of our research demonstrate that it is possible to use a low-carbon and low-cost technology to improve the profitability of irrigated rice crops, which contributes to the sustainability of rice farming activities in Brazil and respects the environment. The use of inoculants combined with the reduction of nitrogen fertilization in irrigated rice, associated with other management practices, can reduce the emission of greenhouse gases and mitigate global climate change. In a review of plant growth-promoting microorganisms (MPCV) and their derived compounds, Naamala and Smith [56] highlighted the need for research to make the use of MPCV technology more effective in developed and developing countries, with the aim to increase plant growth and to reduce greenhouse gas emissions from the agricultural sector. In this sense, in work carried out in the field with genotypes of *Brachiaria* spp., Hungria et al. [32] found that *A. brasilense* strains Ab-V5 and Ab-V6 increased N accumulation in the biomass, which were equivalent to a second application of 40 kg ha^{-1} of fertilizer N. At the same time, they proved that gains of 0.103 Mg C ha^{-1}

were due to inoculation, which corresponded to 0.309 Mg CO_2 eq. ha^{-1}. Thus, the authors concluded that inoculation with *A. brasilense* may represent a key component in the pasture recovery program and help sequester CO_2 from the atmosphere. In turn, Veçozzi et al. [57], in a study carried out with greenhouse gas emissions associated with controlled-release nitrogen fertilizer and urea in irrigated rice cultivation, determined that the management of nitrogen fertilization influenced the partial global warming potential. The determined values corresponded to 13,946 kg CO_2 eq. ha^{-1} for urea, 14,297 kg CO_2 eq. ha^{-1} for the controlled release nitrogen fertilizer applied by surface broadcast, and 11,683 kg CO_2 eq. ha^{-1} for controlled release nitrogen fertilizer applied locally in the sowing furrow.

Nitrogen use efficiency (NUE) by rice use is necessary for environmental conservation and agricultural sustainability. Genetically modified crops and integrated management practices are the most effective biotechnological methods for enhancing NUE [58]. The development of new cultivars that utilize N more efficiently through conventional breeding, as well as through the use of CRISP-based gene editing, such as manipulation of the flavone biosynthetic pathway, must be considered as a viable strategy for the induction of biological fixation in rice and a reduction in the use of inorganic nitrogen fertilizers [59].

Thus, we emphasize that although the use of the inoculant *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) in irrigated rice does not completely replace the application of mineral nitrogen fertilizers when aiming at high yields, it may reduce the amount demanded of this nutrient and, thus, reduce the global warming potential.

5. Conclusions

Based on experiments carried out with irrigated rice during five agricultural seasons under different soil and climatic conditions, we concluded that the inoculation of *Azospirillum brasilense* (strains Ab-V5 + Ab-V6) combined with nitrogen fertilization with reduced coverage (90 kg of N ha^{-1}) promoted an average increase of 30% in rice production when compared to the absence of nitrogen fertilizer. In our results obtained in five agricultural seasons, the inoculation of *A. brasilense* with 90 kg of N ha^{-1} compensated for the reduction of 30 kg of N ha^{-1} of the topdressing fertilization, and provided the same productive level of the complete mineral nitrogen fertilization without inoculation. In addition, different cultivars evaluated in the validation areas in lowlands presented rice yields in the presence of nitrogen fertilizer combined with *Azospirillum*, with or without mineral N reduction, that were equal to or higher than the complete nitrogen fertilization, as well as above the productivity average of this cereal in the State of RS, Brazil. The results of this study demonstrate that inoculation with *Azospirillum* is equivalent to an additional application of 30 kg of N ha^{-1} and that inoculants composed of *A. brasilense* (strains Ab-V5 and Ab-V6) promote an increase in the efficiency of N use of a mineral source in irrigated rice cultivation to provide a better agronomic and productive performance of the culture. Thus, the consistent results of agronomic efficacy in the field attest to the recommendation and viability of the inoculant evaluated, which is based on *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) as a growth promoter in rice.

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