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Article

Coinoculation with Growth-Promoting Bacteria Increases the Efficiency of Nitrogen Use by Irrigated Rice

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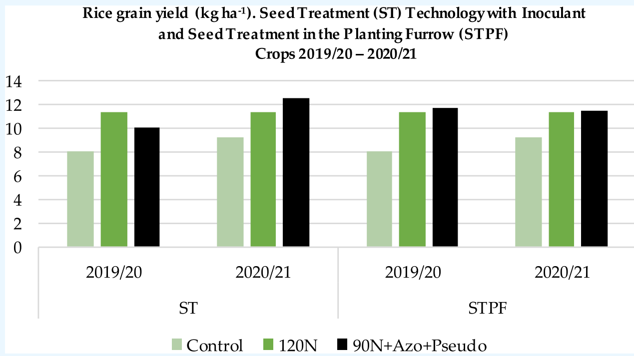
ABSTRACT: Considering the importance of rice (*Oryza sativa* L.) for global food and its significant production in Brazil, strategies for its sustainable production are focused on technologies to increase productivity and decrease the use of chemical nitrogen fertilizers. An alternative for this is the use of plant growth-promoting bacteria that have proven to be efficient for increasing production and nutrient promotion in cereals. This study reports the use of coinoculation with *Azospirillum brasilense* and *Pseudomonas fluorescens* to inoculate irrigated rice through seed treatment (ST) with inoculant and seed inoculation in planting furrow technology (PFT) in four field experiments. The inoculation technologies increased rice yields in the presence of *A. brasilense* + *P. fluorescens* and with a reduction in mineral N (30 kg of N ha^{-1}), equal to or greater when nitrogen fertilizer was present alone. Our results demonstrate that coinoculation with *A. brasilense* (strain Ab-V6) and *P. fluorescens* (strain CCTB03) increases the efficiency of N use from a mineral source in irrigated rice, with an increase of 37% in economic production (grains per unit of N applied), providing better agronomic performance of the crop.



■ INTRODUCTION

The benefits of seed inoculation with *Azospirillum brasilense* on the growth and yield of different grasses have been shown in numerous studies.[1−5] A recent example is in Brazil, where the inoculation of irrigated rice with the bacteria *A. brasilense*, strains Ab-V5 and Ab-V6, used in a commercial inoculant, makes it possible to combine it with mineral nitrogen (N) through seed inoculation technology, resulting in yield increases equal to or higher than those obtained with 100% mineral N.[6] Inoculation combined with N allows a reduction of 30 kg ha^{-1} of mineral N, maintaining the same productive level as the BRS Pampa CL cultivar with complete mineral nitrogen fertilization.[6] In Brazil, there is proof of fulfillment of an inoculant containing strains of *A. brasilense* for irrigated rice with cultivars BRS Pampa and BRS Pampa CL.[6,7] In addition, the commercial inoculant containing *A. brasilense* combined with reduced nitrogen fertilization increases the producer's net income and provides a greater productive performance of the BRS Pampeira cultivar, and when combined with producer-standard nitrogen fertilization, it increases the producer's net income and provides a greater yield performance of BRS Pampa and IRGA 424RI cultivars.[8]

In Argentina, there is also evidence that *A. brasilense* isolates promote the growth of different genotypes of rice plants with reduced nitrogen fertilization, as evidenced by the significant increase in plant height, root length and, in some cases, aerial dry mass.[9]

In addition to *Azospirillum*, several genera of rhizospheric bacteria can provide benefits for the promotion of rice growth, such as some *Pseudomonas* species, plant growth-promoting bacteria (PGPB) and disease biocontrol agents.[10] Among these, *Pseudomonas fluorescens* stands out for its high metabolic capacity, which makes it multifunctional in addition to this favorable aspect. This species has the desirable characteristics of phosphate solubilization, production of enzymes that promote plant growth and hormones and antagonism against pathogenic fungi that affect various crops.[11] In grasses, *P. fluorescens* produces indole acetic acid (IAA) in response to root exudates.[12] In Brazil, the inoculation of maize seeds with *P. fluorescens* increases grain productivity by approximately 31% with a 25% reduction in nitrogen fertilization, concomitantly allowing a reduction in costs and less environmental impact.[13] In addition, inoculation of *P. fluorescens* associated with sowing fertilization with NPK favors the development and productive performance of second-crop corn.[14]

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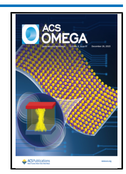

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Table 1. Chemical and Particle Size Properties and Population of Diazotrophic Bacteria in the Soil of the Experimental Area[a]

crop	pH	Al	H + Al	BS	Ca	Mg	Na	K	P	OM, %	ECEC	clay, %	DB, CFU g^{-1} soil
	water (1:1)	cmol$_c$ dm^{-3}					mg dm^{-3}						
2016/17	5.5	0.0	2.7	56	2.8	1.1		27	37	1.2	4.3	20	1.4×10^5
2017/18	5.7	0.0	2.0	72	2.9	1.9	36	45	60	1.4	5.2	21	2.0×10^5
2019/20	5.0	0.4	3.6	60	3.5	1.7	30	70	42	1.6	9.2	26	7.6×10^5
2020/21	5.3	0.3	2.8	69	3.2	2.7	32	70	25	1.3	6.5	20	8.1×10^6

[a]ECEC = effective cation exchange capacity; BS = base saturation; OM = organic matter; DB = diazotrophic bacteria.

In rice, *P. fluorescens* is efficient as a biocontrol agent for a fungus (*Rhizoctonia solani*) that is transmitted through the soil and causes sheath blight,[15] and it also reduces the severity of blast disease (*Magnaporthe oryzae* B. Couch) [*Pyricularia grisea* (Cooke) Sacc.] on rice leaves.[16] Furthermore, the combined inoculation of two PGPB modifies the microbial community of the rice rhizosphere, promotes plant growth, and improves grain yield and soil health.[10] Coinoculation of the rice rhizosphere with *A. brasilense* and *P. fluorescens* or inoculation with *P. fluorescens* alone increases ammonification activity in the soil, mineralized N content in the soil, and nitrogenase activity in the rice rhizosphere, resulting in increased production of root and shoot biomass due to the potential supply of N to rice plants.[17]

Given the importance of rice (*Oryza sativa* L.) for world food, considering that the world production of this cereal is on the order of 512.6 million tons (processed basis),[18] and in Brazil, an estimated production of the 2022/23 season of approximately 10,169.3 million tons,[19] strategies for sustainable production of this grain are focused on technologies to increase productivity and reduce the use of chemical fertilizers.[20] An alternative is the use of PGPB, which increase the growth and productivity of irrigated rice cultivated in the State of Rio Grande do Sul (RS),[6] where production is concentrated in Brazil, with approximately one million hectares cultivated and an annual production of over 7 million tons.[19] However, the promotion of irrigated rice growth with the use of *A. brasilense* combined with *P. fluorescens* by coinoculation technology has not been employed, and the present work represents the first report on coinoculation with PGPB in flood-irrigated rice in lowlands.

In this context, the object of this study was to evaluate the combination of *A. brasilense* (Ab-V6) and *P. fluorescens* (CCTB03) strains inoculated in irrigated rice in the technologies of seed treatment with inoculant and seed inoculation in the planting furrow, resulting in increased productivity with reduced nitrogen fertilization and increased efficiency in the use of nitrogen by rice in the edaphoclimatic conditions of RS, Brazil.

■ MATERIALS AND METHODS

Edaphoclimatic Characteristics. The experiments were installed in the 2016/17, 2017/18, 2019/20, and 2020/21 agricultural seasons, under Planosol Haplico, in the field of the Experimental Station Terras Baixas (ETB) (Latitude: 31° 52′ 00″ S; Longitude: 52° 21′ 24″ W), municipality of Capão do Leão, RS. In this region, southern Brazil, the local climate is classified as subtropical (Cfa − Köppen),[21] with an average annual precipitation and temperature of 1367 mm and 17.8 °C, respectively.[22]

■ RESEARCH FIELD EXPERIMENTS

Soil Analysis. To assess soil fertility, before implementing the experiments, samples were collected at a depth of 0−20 cm, with chemical analyses carried out in an Official Network of Soil and Plant Tissue Analysis Laboratories of the States of Rio Grande do Sul and Santa Catarina (ROLAS) certified laboratory. Soil samples were collected at a depth of 0−10 cm before the implementation of the experiments to estimate the natural abundance of diazotrophic microorganisms using the most probable number method (MPN) in semisolid NFb culture medium.[23] Data from the chemical, granulometric, and population analyses of diazotrophic bacteria are shown in Table 1.

Phytotechnical Treatments and Practices. Two seed inoculation technologies were used in agricultural seasons: seed treatment (ST) [2016/17 and 2017/18] and seed treatment in the planting furrow (STPF) [2019/20 and 2020/21]. Topdressing nitrogen fertilization was applied in installments at the stages of three to four leaves (V3−V4) and panicle initiation (R0). In the first coverage with N, 60% of the expected N dose was applied, and in the second, the remaining 40%. The first coverage with N was carried out on dry soil, immediately before the start of irrigation by flooding the soil, and the second coverage was carried out on noncirculating water. Dates of topdressing nitrogen fertilization were estimated using the degree-day method.[24] The evaluations of the experiments included the determination of the foliar nitrogen level, the agronomic and productive performance of the culture, the chlorophyll index, and the accumulation of N in the aerial part of the plants and exported by the rice grains. The variable relative chlorophyll index (CRI) and N content in the rice flag leaf measured the N level in the plant. The CRI was measured with a SPAD-502 Minolta chlorophyll meter on the flag leaf of 10 plants per experimental plot 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.[25] The nitrogen exported by the grains was measured in samples taken from the production area (4.90 m^2). The grain mass variable was measured at the harvest maturation stage (stage R9) in a single plot consisting of seven rows of plants 4 m in length, correcting the data to 130 g kg^{-1} moisture.

Strains and Inoculant. The *A. brasilense* strain Ab-V6 and *P. fluorescens* strain CCTB03 are components of a commercial inoculant called BIOFREE (liquid formulation), produced by Biotrop, from Curitiba, PR, Brazil, which is registered in the Ministry of Agriculture and Livestock and Supply (MAPA) (No. PR001593-8.000001) as a growth promoter, with a guaranteed minimum concentration of 1.0×10^{11} CFU L^{-1}.

The doses applied to the inoculant in the tests were as follows: (1) seed treatment = 100 mL of 50 kg^{-1} seeds; and (2) seed treatment in the sowing soil = 300 mL ha^{-1} and an

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Table 2. Shoot Dry Matter Production of Rice Plants, Nitrogen Content Accumulated in the Aerial Part of Rice Plants, Amount of Nitrogen Exported by the Grains, and Rice Grain Yield[a]

treatments	shoot dry matter production (kg ha^{-1})	nitrogen content accumulated in the aerial part of rice plants (%)	amount of nitrogen exported by the grains (%)	rice grain yield (kg ha^{-1})
		2016/17		
control	6.800 Ac	0.56 Ab	0.79 Ac	9206 Ab
120N[b]	11.750 Ab	1.00 Aa	1.07 Ab	11,686 Ab
110N + azo + pseudo[c]	18.500 Aa	0.97 Aa	1.16 Ba	12,170 Aa
C.V. (%)	<10			
		2017/18		
control	5.570 Bc	0.55 Ab	0.85 Ac	8913 Ab
120N[b]	9.480 Bb	0.95 Aa	1.05 Ab	12,229 Aa
110N + azo + pseudo[c]	19.420 Aa	0.95 Aa	1.94 Aa	12,932 Aa
C.V. (%)	<10			

[a]Averages followed by the same letter, lowercase in columns (compare treatments) and uppercase in rows (compare harvests), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. The experiments were carried out with the technology of seed treatment with inoculant (ST). Crops 2016/17 to 2017/18. [b]120 kg of N ha^{-1}. [c]110 kg of N ha^{-1} + *A. brasilense* and *P. fluorescens*.

additive (Protege TS). The doses applied in the trials were 100 and 300 mL of the product for treatments with seed inoculation (50 kg) and in the planting furrow, respectively. An additive Protege TS (Biotrop, Curitiba, PR, Brazil) was also applied at a dose of 100 mL of 50 kg^{-1} of seeds to guarantee a better coating of the seeds to improve seed coating with the inoculant.

Field Experiments in Crop 2016/17 and 2017/18. The experiments were carried out using rice seed treatment (TS) technology with the inoculant [*A. brasilense* (strain Ab-V6) and *P. fluorescens* (strain CCTB03)] in the liquid formulation. The treatments were arranged in a randomized block design with four repetitions of experimental plots with dimensions of 1.58 × 5.0 m^2, nine lines, and spacing between lines of 17.5 cm, being individualized by rammed earth. The cultivar BRS Pampa, with an early cycle, with 118 days from emergence to complete grain maturation,[26] and with wide adaptation in RS, was used at a density of 90 kg ha^{-1} of seeds and sown under conventional tillage. In the base fertilization, 300 kg of ha^{-1} of formulations 00-25-25 (treatment without inoculation and without N) and 05-25-25 (treatment with inoculation and reduced N) were applied in a localized manner in the planting furrow. The treatments comprised (1) full control (without N and without *A. brasilense* and *P. fluorescens*); (2) full nitrogen fertilization [(120 kg ha^{-1} = 20 kg of N in the base fertilization + 100 kg of N ha^{-1}) by dressing (divided in V3V4 (60 kg of N ha^{-1}) and R0 (40 kg of N ha^{-1}))] and without inoculation with *A. brasilense* and *P. fluorescens*; (3) combination of reduced nitrogen fertilization [(110 kg ha^{-1} = 20 kg of N in the base fertilization + 90 kg of N ha^{-1}) by dressing (divided in V3V4 (42 kg of N ha^{-1}) and R0 (28 kg of N ha^{-1}))] and inoculation with *A. brasilense* and *P. fluorescens*.

Field Experiments in Crop 2019/20 and 2020/21. The experiments were carried out using rice seed treatment (ST) and rice furrow inoculation (STPF) technologies with the inoculant [*A. brasilense* (Ab-V6 strain) and *P. fluorescens* (CCTB03 strain)] in the liquid formulation. The treatments were arranged in strips with dimensions of 100 × 4.42 m^2, constituting the experimental units, individualized by rammed earth walls.

The cultivar BRS Pampa CL, with an early cycle, with 118 days from emergence to complete grain maturation,[26] with wide adaptation in RS, was used at a density of 100 kg ha^{-1} of seeds and sown under conventional tillage. In the base fertilization, 300 kg of ha^{-1} of the 05-20-20 formulation was used and applied locally in the planting furrow. As a complement to the predicted potassium dose, 45 kg of ha^{-1} KCl was applied by broadcasting before sowing. The volume of spray used in the treatments with inoculation in the rice planting furrow was 120 L ha^{-1}. The equipment used was an HUNTER 1200 (Orion, Pompéia, SP, Brazil) tank (600 L) for the application of inoculants in the furrows of agricultural crops. The treatments comprised (1) absolute control (without N and without *A. brasilense* and *P. fluorescens*); (2) complete nitrogen fertilization [(20 kg of N in base fertilization +100 kg of N ha^{-1}) in topdressing (divided into V3V4 (60 kg of N ha^{-1}) and R0 (40 kg of N ha^{-1}))] and without *A. brasilense* and *P. fluorescens*; (3) reduced nitrogen fertilization [(20 kg of N in the base fertilization +70 kg of N ha^{-1}) in topdressing installed in V3V4 (54 kg of N ha^{-1}) and R0 (36 kg of N ha^{-1})] and inoculation with *A. brasilense* and *P. fluorescens*.

Statistical Analysis. The results of the 2016/17 and 2017/18 harvests were analyzed together. In the other harvests (2019/20 and 2020/21), individual analyses were carried out. The data were submitted to the normality test of the variables and homogeneity and analysis of variance (ANOVA) with 95% confidence. When the F value was significant, the means were compared using Tukey's test ($p < 0.05$). All analyses were performed with the statistical software R.[27]

RESULTS

Seed Treatment (ST) Technology with Inoculum—Agricultural Seasons 2016/17 and 2017/18. In the absence of nitrogen and inoculant fertilization, there was a lower dry matter production and a significant difference between the harvests (Table 2). The inoculant *A. brasilense* + *P. fluorescens* combined with a dose of 110 kg of N ha^{-1} showed the highest average mass accumulation over the two agricultural seasons (18,960 kg ha^{-1}). The dry matter production with the use of *A. brasilense* + *P. fluorescens* exceeded that provided by the use of the dose of 120 kg ha^{-1} of N, recommended for rice, resulting in an increase of 78.6%. This result demonstrates the potential of this inoculant to promote rice plant growth and possibly biological nitrogen fixation (BNF). In turn, *P. fluorescens* (strain CCTB03) also determines a significant increase in the biomass of maize plants.[13]

The effect of inoculation with a reduced dose of N (110 kg ha^{-1}) occurred with equal amounts of N accumulated in rice plants compared to the recommended dose of N (120 kg ha^{-1}) in both seasons (Table 2). The lowest average accumulation of N (0.55%) was observed in the absence of nitrogen fertilization, while the highest amount of accumulated N (1%) in rice plants was in the presence of 120 kg ha^{-1} of N (2016/17 season). These results show that, in general, the use of the inoculant *A. brasilense* + *P. fluorescens* promotes a significant increase in the accumulation of N in the stems and leaves of rice plants.

The N content exported by the grains with the use of the inoculant and the dose of 110 kg of N ha^{-1} differed from the treatment with the dose of 120 kg of N ha^{-1} in both harvests (Table 2). The inoculant *A. brasilense* + *P. fluorescens* promotes the growth of the root system of the plants, which, consequently, creates conditions to increase the absorption of N that will be translocated to the grains. The inoculant *A. brasilense* + *P. fluorescens* combined with 110 kg of N ha^{-1} promoted an average increase of 93% in the content of N exported by the grains compared to the dose of 120 kg of N ha^{-1} *P. fluorescens*, which also enabled the accumulation of nitrogen (protein) in wheat kernels.[28] The inoculation of wheat seeds with a combination of *Azospirillum* + *Pseudomonas* and the application of N sources increased the assimilation of nitrogen by wheat grains, which resulted in an increase in production.[29]

The average productivity values of the two agricultural seasons, presented in Table 2, show significant effects of the treatments with the use of the inoculant A. brasilense + P. fluorescens on rice productivity. Yield decreases in the control that has no nitrogen in coverage, tillering and reproductive phases of the crop, when nitrogen requirements are higher and rice plants are more efficient in absorbing N for grain production.[30] The dose of 90 kg of N ha^{-1} combined with the liquid inoculant *A. brasilense* + *P. fluorescens* showed higher productivity (12,170 kg ha^{-1}) than the dose of 100 kg of N ha^{-1} N in coverage (11,686 kg ha^{-1}) in the 2016/17 harvest. In turn, in the 2017/18 season, there was no distinction between treatments with doses of 100 and 90 kg of N ha^{-1} in coverage, indicating the performance of the inoculant *A. brasilense* + *P. fluorescens* to provide high grain productivity of rice with a reduction of 10 kg of N ha^{-1} in coverage. It is also noted that the inoculant *A. brasilense* + *P. fluorescens* increased the productivity of irrigated rice by 5% in both agricultural seasons, and compared to the noninoculated control and with the absence of nitrogen fertilization, the increase was 38.5%. In work carried out in a field with a maize crop associated with a reduction in nitrogen fertilization of 75%, *P. fluorescens* promoted a grain yield equivalent to 100% nitrogen fertilization, resulting in profitability for the producer and a reduction in environmental impact.[13] Thus, inoculation of rice seeds with *A. brasilense* + *P. fluorescens* allows the removal of 10 kg of N ha^{-1} in topdressing without impairing grain yield.

Seed Treatment (ST) Technology with Inoculant and Seed Treatment in the Planting Furrow (STPF)—Agricultural Seasons 2019/20 and 2020/21. *Seed Treatment in the Planting Furrow (STPF).* Rice productivity was significantly increased with the use of the inoculant *A. brasilense* + *P. fluorescens* (Table 3). In the control, the average grain yield was 8679 kg ha^{-1}, which was different from the other treatments. At a dose of 120 kg of N ha^{-1}, the average increase in productivity was 31% compared to the control. At the dose of 90 kg of N ha^{-1}, the average increase in productivity was 34% compared to the control. Under the dose of 90 kg of N ha^{-1} with *A. brasilense* + *P. fluorescens*, in the application of the inoculant in the planting furrow with a spray volume of 120 L ha^{-1}, a productivity increase of 2.6% was obtained compared to the 120 kg of N ha^{-1}. In the syrup volume (120 L), the inoculant *A. brasilense* (1×10^{10} CFU mL^{-1}) + *P. fluorescens* (1×10^{9} CFU mL^{-1}) provided a higher grain yield (11,774 kg ha^{-1}). These results indicate that the performance of the inoculant in the inoculation technology in the rice furrow is enhanced by the high concentration of bacterial cells in a spray volume of 120 L ha^{-1}. Thus, the effect of the dilution of the inoculant on the evaluated spray volume is one of the factors that must be considered in the adjustment of the inoculation technology in the rice planting furrow. We also emphasize the importance of liquid formulations of inoculants with a high concentration of cells for greater dissolution in the syrups. Several studies have demonstrated the positive effect of inoculation in planting furrow technology for the coinoculation of soybeans and, more recently, for rice,[31] highlighting the advantages of the technique for inoculation with diazotrophic bacteria and growth promoters, such as the promotion of root growth, resulting in greater absorption of water and nutrients from the soil, greater production of aerial part dry matter, greater plant population, number of tillers and panicles, greater productive performance of rice cultivars and, consequently, greater net income for the rice grower.

Table 3. Rice Grain Yield, Relative Chlorophyll Index, and Aerial Part Dry Matter Production[a]

treatments	rice grain yield (kg ha^{-1})	relative chlorophyll index	aerial part dry mass (kg ha^{-1})
	2019/20		
control	8100 c	33 b	6660 b
120N[b]	11,349 ab	34 b	9580 a
90N + azo + pseudo[b]	11,774 a	42 a	9560 a
C.V. (%)	<10		
	2020/21		
control	9258 b	44 b	6600 b
120N[b]	11,375 a	44 b	9840 ab
90N + azo + pseudo[c]	11,564 a	46 a	9940 a
C.V. (%)	<10		

[a]Averages followed by the same letter, lowercase in columns (compare treatments), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. The experiments were carried out with the technology of seed treatment with an inoculant in the planting furrow (STPF). Crops 2019/20 to 2020/21. [b]120 kg of N ha^{-1}. [c]90 kg of N ha^{-1} + *A. brasilense* and *P. fluorescens*.

For the relative chlorophyll index (RCI) variable, there was a significant effect of *A. brasilense* + *P. fluorescens* with 90 kg of N ha^{-1} (Table 3). In general, the use of inoculant provided a higher RCI than the dose with complete nitrogen fertilization (120 kg of N ha^{-1}) (12.8%) and the noninoculated control (14.2%) (Table 3).

The inoculant *A. brasilense* + *P. fluorescens* combined with 90 kg ha^{-1} had a marked effect on the dry matter production of the aerial part of the rice plants in the two agricultural seasons, with an average of 9750 kg ha^{-1} (Table 3). It is noteworthy that the dry mass production of rice achieved by using the inoculant with reduced nitrogen fertilization was equivalent to that provided by the dose of 120 kg of N ha^{-1}.

Seed Treatment (ST) Technology with Inoculum. Table 4 shows the productivity values for the 2019/20 and 2020/21 agricultural seasons. For the seed treatment with the inoculant, high grain yields were again obtained with a dose of 90 kg of N ha^{-1}, providing an increase of approximately 30% with *A. brasilense* + *P. fluorescens* compared to the control. It is also noted that there was only an average difference of 0.4% of the positive control (100% N) compared to the dose of 90 kg of N ha^{-1} with *A. brasilense* + *P. fluorescens*, which was not significant considering the coefficient of variation. In addition, the profitability benefit for the producer by reducing 30 kg of N ha^{-1} justifies the use of the inoculant.

Table 4. Rice Grain Yield, Relative Chlorophyll Index, and Aerial Part Dry Matter Production[a]

treatments	rice grain yield (kg ha^{-1})	relative chlorophyll index	aerial part dry mass (kg ha^{-1})
	2019/20		
control	8100 c	33 b[b]	6660 b
120N[b]	11,349 a	34 b	9580 a
90N + azo + pseudo[c]	10,086 ab	34 b	6520 b
C.V. (%)	<15		
	2020/21		
control	9258 c	44 b	6600 b
120N[b]	11,375 b	44 b	9840 a
90N + azo + pseudo[c]	12,548 a	43 b	7060 b
C.V. (%)	<15		

[a]Averages followed by the same letter, lowercase in columns (compare treatments), do not differ significantly from each other by Tukey's test at $p \leq 0.05$. Coefficient of variation [CV (%)]. The experiments were carried out with the technology of seed treatment with inoculant (ST). Crops 2019/20 to 2020/21. [b]120 kg of N ha^{-1}. [c]90 kg of N ha^{-1} + *A. brasilense* and *P. fluorescens*.

Similar results determined that inoculation with plant growth-promoting bacteria may represent a viable economic and environmental strategy to improve pasture production,[3] confirming that *A. brasilense* increases the aboveground part biomass by 16.8% with inoculation by spraying seeds and leaves, while *P. fluorescens* increases by 15.2% with the addition of 40 kg of N ha^{-1}.[3] The benefits were attributed to improvement in root architecture by phytohormone synthesis, as well as biological nitrogen fixation and P acquisition (solubilization of phosphate and siderophore synthesis) by *A. brasilense* and ACCdeaminase by *P. fluorescens*, which also contributed to plant growth and nutritional status.[3] However, another study reported that *P. fluorescens* was responsible for the greater production of marandu grass roots (*Urochloa ruziziensis*) and was more effective for variables such as aerial part dry mass production and total chlorophyll content.[32]

In general, in the 2019/20 season, the use of inoculant with 90 kg of N ha^{-1} increased the RCI compared to the noninoculated control (Table 4). In the second season (2020 and 2021), there was also no significant difference between N doses on the RCI variable. The comparison between the treatments' N doses without inoculant and the reduced N dose with the inoculant *A. brasilense* + *P. fluorescens* shows that, in general, the use of the inoculant promotes an increase in RCI. For the dry matter production variable of the aerial part of the rice plants (Table 4), the combination of 90 kg of N ha^{-1} with the inoculant *A. brasilense* + *P. fluorescens* responded with an average shoot dry mass production of 6790 kg ha^{-1}. The lower production of dry mass of stems and leaves (aerial part dry mass) provided by the use of 90 kg of N ha^{-1} with the inoculant *A. brasilense* + *P. fluorescens* was not reflected, however, in the productive performance of the rice cultivar BRS Pampa CL (Table 4).

The agronomic efficiency (AE), which means the economic production obtained (grains, in the case of annual crops) per unit of applied nutrient, can be calculated by the following equation:[33]

$$\text{AE} = [(\text{production in kg with fertilization}) - (\text{production in kg without fertilization})] / [\text{amount of nutrient applied in kg}] \quad (1)$$

The values of AEs in the use of N needed by irrigated rice for grain production, defined in eq 1, considering the combined use of *A. brasilense* and *P. fluorescens* with reduced nitrogen fertilization and complete nitrogen fertilization, in the TS and TSSP technologies, are presented in Figures 1 and 2. Thus, the agronomic efficiency (AE) in the use of N by irrigated rice with *A. brasilense* (strain Ab-V6) and *P. fluorescens* (strain CCTB03) combined with reduced nitrogen fertilization (110 and 90 kg of N ha^{-1}) was an average of 31 kg grains kg^{-1} of N applied in the ST and STPF technologies (Figure 1). The AE for 120 kg of ha^{-1} was 23 kg grains kg^{-1} N applied, and the greatest percentage contribution to the increase in grain production was in the crop seasons 2016/17 (29%) and 2017/18 (30%) (Figure 2). These results also indicate that there was economic efficiency[33] with the reduction of N and the use of coinoculation of rice in ST and STPF technologies.

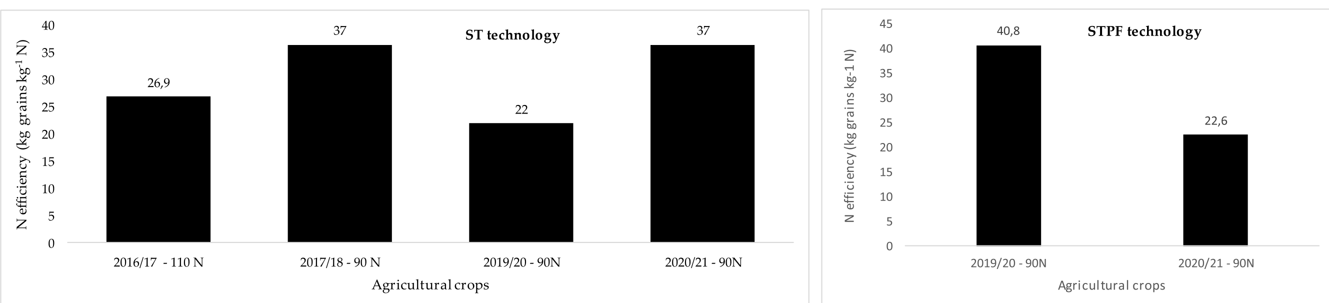

Figure 1. Agronomic efficiency (AE) of the irrigated rice cultivar BRS Pampa CL as a function of *A. brasilense* + *P. fluorescens* combined with N in ST (110 and 90 kg of N ha^{-1}) and STPF (90 kg of N ha^{-1}) technology. Crop seasons 2016/17, 2017/18, 2019/20, and 2020/21.

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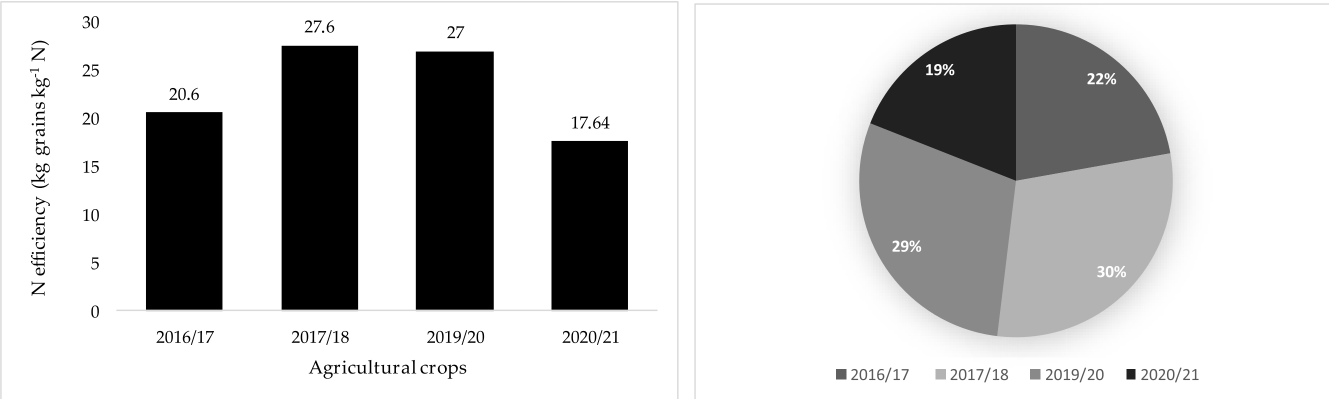

Figure 2. Agronomic efficiency (AE) of the irrigated rice cultivar BRS Pampa CL as a function of complete nitrogen fertilization (120 kg of N ha^{-1}) and the percentage contribution to increasing grain production in four crop seasons.

DISCUSSION

In Brazil, flood-irrigated rice cultivation is cultivated in 1.3 million hectares of lowland, producing ±12 million tons of cereal annually.[19] Flood-irrigated rice, concentrated in southern Brazil, provides ±70% of national production.[7] In the South 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.[7]

The FBN is part of the Sectorial Plan for Mitigation and Adaptation to Climate Change for the Consolidation of an Economy with Low Carbon Emissions in Agriculture, created by the Ministry of Agriculture, Livestock, and Supply (public policy). FBN in rice, in addition to the agronomic potential for saving approximately 30% of N,[6] involves inputs of a biological nature that benefit the promotion of plant root growth and greater resistance to biotic and abiotic stresses. In addition, diazotrophic plant growth-promoting bacteria can improve the root system of rice plants and consequently increase N assimilation from the soil.

In this study, in four agricultural seasons, average yields of more than 12,000 kg ha^{-1} of the BRS Pampa CL cultivar were obtained with the combined use of *A. brasilense* + *P. fluorescens* and reduced mineral nitrogen fertilization. The productivity differential of the combination of 90 kg of N ha^{-1} (top dressing) with the inoculation in relation to the 120 kg of N ha^{-1} treatment was 5%, corresponding to approximately 11 bags ha^{-1}. Considering that the price of paddy rice (50 kg bag) is R$ 69.04 (May 2022),[34] there is a gain of R$ 759 ha^{-1} with the differential of bags obtained with the use of *A. brasilense* (strain Ab-V6) + *P. fluorescens* (strain CCTB03).

For environmental impacts, with the rationalization of the use of chemical nitrogen fertilizer, there is less potential for contamination of water resources, gaseous losses (N_2O) and susceptibility of irrigated rice cultivars to fungal diseases, mainly blast. We also highlight the importance of FBN and the promotion of growth in irrigated rice for organic systems, where there is a need for alternatives for the supply of nitrogen, constituting a critical aspect regarding the fertilization of this crop.[35] The use of inoculant is a sustainable way of supplying N and promoting growth to crops, in addition to mineral nitrogen fertilization, and several genera of plant growth-promoting bacteria (PGPB), mainly *Azospirillum* and *Pseudomonas* combined in one inoculant, is a new strategy for this purpose.[36]

Thus, coinoculation of rice in ST and STPF technologies contributes to low-carbon agriculture and cost and, consequently, profitability of irrigated rice crops, intensifying the sustainability of rice activities in Brazil. Thus, genotypes of *Urochloa* spp. inoculated with the Ab-V5 and Ab-V6 strains of *A. brasilense* increase the accumulation of N in the biomass, being equivalent to the application of 40 kg ha^{-1} of N fertilizer, and the inoculation factor contributes to the sequestration of CO_2 from the atmosphere (0.309 Mg CO_2 equiv ha^{-1}).[2] In turn, the management of nitrogen fertilization influences the partial global warming potential, and the use of controlled release nitrogen fertilizer applied locally in the rice sowing furrow determines lower values of CO_2 release (11,638 kg CO_2 equiv ha^{-1}).[37]

In this way, despite the use of the inoculant *A. brasilense* + *P. fluorescens* in irrigated rice, not completely avoiding the application of mineral nitrogen fertilizers could contribute to reducing costs and mitigating global warming. Furthermore, to improve the environmental quality and soil fertility deteriorated by excessive N application, coinoculation of rice seedlings with a mixture of *A. brasilense* and *P. fluorescens* can be an alternative measure to improve the N supply potential and rice productivity.[38] Microbial consortia provide numerous benefits for plants, such as the developed consortium (strains *Bacillus subtilis* NM-2, *Brucella hematophila* NM-4, and *Bacillus cereus* NM-6), which showed the efficient ability to alleviate drought stress in the rice genotype with significantly improved grain yield.[39] In the rice intensification system (SRI) with microbial inoculation (*Anabaena*–*Trichoderma viride* and *Anabaema*–*Mesorhizobium* sp.) contributes significantly to the increase in nitrogen and zinc absorption in Basmati rice grains and higher productivity indexes, also illustrating the benefits of a cyanobacterial consortium.[40] In another study with grain culture and coinoculation, it was reported that the use of a microbial inoculant composed of photosynthetic cyanobacteria (*Anabaema cylindrica* and *Nostoc muscorum*) and *A. brasilense*, combined with nitrogen fertilization, favored maize production components.[41]

In summary, we demonstrated that *A. brasilense* (strain Ab-V6) + *P. fluorescens* (strain CCTB03) combined with 70 and 90 kg of N ha^{-1} in coverage promoted average increases of 30 and 38.5%, respectively, in rice production using ST technology. Other authors state that *P. fluorescens* significantly improved the ammonification activities in the rhizosphere when the N application rate was higher than 90 kg of N ha^{-1}

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and that *A. brasilense* greatly improved the N_2-fixation activities in the rhizosphere when the N application rate was lower than 90 kg of N ha^{-1}.[37] This effect was also observed in the TISP with 70 kg of N ha^{-1}, where the productivity increase was 30%. TISP presents itself as an alternative inoculation practice to minimize the negative effects of pesticides applied to seed treatments. The application of inoculant (200 mL ha^{-1}) based on *A. brasilense* in the corn sowing furrow in combination with different doses of nitrogen fertilization proved to be a viable technology for increasing grain production of this cereal in the cerrado biome in Brazil.[42] In another study, similar results were reported for TISP as an alternative method for inoculating cereals (corn and wheat) with *A. brasilense*, reducing or avoiding impacts of chemicals applied in seed treatment and enabling a 25% reduction in nitrogen fertilization and high grain production.[43]

In a recent review based on the world market for inoculants, the use of inoculants containing microorganisms of a "different type" has expanded and the mixed inoculant has combined microorganisms whose major processes are BNF (e.g., *Bradyrhizobium* spp., *Rhizobium* spp.) and phytohormone production (e.g., *Azospirillum* spp., *Pseudomonas* spp.), solubilization of phosphate (e.g., *Bacillus* spp.), or biological control (e.g., *Pseudomonas* spp., *Bacillus* spp.)[44] and, in Brazil, is present in commercial bioinputs for various grain crops. Additionally, other genera of growth-promoting bacteria, such as *B. subtilis* and *Bacillus megaterium*, can also mitigate the deleterious effects of water stress, improving rice growth and productivity under water stress.[45] Inoculation and coinoculation with *Azospirillum* sp. and *Bacillus* sp. in the early development of upland rice and can promote plant growth, especially the root system.[46]

In addition to the economic benefits of coinoculation in irrigated rice (*A. brasilense* + *P. fluorescens*), environmental services are also associated with this technology, given the example of soybeans in Brazil, where the replacement of nitrogen fertilizer with *Bradyrhizobium* spp. and coinoculation with *A. brasilense* corresponds to the mitigation of 183 million $MgCO_2$-e in an agricultural crop, equivalent to €5 billion in carbon credit.[47] Other environmental services are being studied and modeled for the mitigation of greenhouse gases, such as CO_2 sequestration involving various biological, chemical and physical soil processes, such as terrestrial enhanced weathering of alkaline silicate minerals, that can generate in the soil an amount of inorganic carbon (SIC) currently estimated to be 720−950 Gt of C, considering the fundamental role of the dynamics of the microbial community in this environmental matrix.[48]

Thus, the agronomic efficiency (AE) in the use of N by rice irrigated with *A. brasilense* (strain Ab-V6) + *P. fluorescens* (strain CCTB03) combined with reduced nitrogen fertilization was 32 kg grains kg^{-1} of N applied, while that for 120 kg of N ha^{-1} was 23 kg grains kg^{-1} of N applied in seed treatment technology (ST) with inoculant. Based on the results of four agricultural seasons with the ST technology, inoculation with *A. brasilense* and *P. fluorescens* combined with 90 and 110 kg of N ha^{-1} compensated for the reduction of 30 and 10 kg ha^{-1} of mineral N from topdressing fertilization, respectively, providing the same productive level of complete mineral nitrogen fertilization (120 kg of N ha^{-1}) without inoculation.

■ CONCLUSIONS

The evaluated inoculation technologies (ST and STPF) allow an increase in rice production with a reduction in mineral N combined with *A. brasilense* + *P. fluorescens*, equal to or higher when nitrogen fertilizer is present alone as well as above the average productivity of this cereal in RS, indicating the benefit of inoculation for greater profitability of the rice grower. Our results demonstrate that coinoculation with *A. brasilense* (strain Ab-V6) and *P. fluorescens* (strain CCTB03) promotes an increase in the economic production of irrigated rice by 37% (grains per unit of applied nitrogen), providing better crop agronomic performance.

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Notes

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