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Pre-inoculation of corn and soybean seeds with Biofix Azos: Assessing viability, survival, and agronomic efficiency

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Abstract: New technologies, such as pre-inoculation with protective additives have been developed to optimize the inoculation of beneficial bacteria like *Azospirillum brasilense* in crops, and represents a promising strategy to optimize the use of bio inputs and streamline field operations. This study evaluated the efficacy of inoculants of pre-inoculating corn seeds with Biofix Azos and soybean seeds with Biofix Azos and Biofix Protec, both performed 30 days before sowing. Four field trials were conducted in different edaphoclimatic regions of Brazil (GO, MG, SP, and PR) during the 2022-2023 season, to ensure greater reliability of the results. Standardized agronomic protocols, randomized block design, and statistical analyses were applied. The treatments of soybean and corn included seed pre-inoculation with Biofix Azos 30 days before sowing, compared to inoculation performed on the day of sowing, fertilization with 200 kg/ha of nitrogen, and a control without fertilization or inoculation. In corn, pre-inoculation with Biofix Azos demonstrated an increase in shoot dry mass, chlorophyll content, and grain nitrogen content, resulting in grain yield gains ranging from 2.8 to 7.7%, compared to the treatment inoculated on the sowing day. In soybeans, co-inoculation with Biofix Azos and Biofix Protec significantly increased nodulation, nodule dry mass, and nitrogen fixation, resulting in grain yield gains ranging from 6.0 to 7.6%, equivalent to the treatment inoculated on the sowing day. The results from pre-inoculation were equal to or superior to those achieved with inoculation on the sowing day. Laboratory tests confirmed the feasibility of pre-inoculation, demonstrating that bacteria maintained their viability for up to 30 days after treatment, even when combined with chemical products. These results indicate that pre-inoculation with Biofix Azos (corn) and Biofix Azos and Biofix Protec (soybean) is an effective strategy to increase grain yield and enhance the sustainability of corn and soybean crops, offering consistent benefits to farmers.

Keywords: Pre-sowing; *Glycine max*; *Zea mays L.*; Seed treatment; Symbiosis; Crop yield.

Abbreviations: CFU_colony-forming unit, FCI_Falker chlorophyll index, DMAP_dry matter of the aerial part, GY_grain yield, RG_relative gain, WTG_weight of a thousand grains, PNG_percentage of nitrogen in grains, TNCG_total nitrogen content in grains, NNMR_number of nodules on the main root, DMNMR_dry mass of nodules on the main root, N_nitrogen, BNF_biological nitrogen fixation.

Introduction

Corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merrill) are two of the most important crops in global agriculture, playing vital roles in food production, livestock feed, and industrial applications. In Brazil, these crops are not only options for agricultural production but also key drivers of economic growth, thanks to their extensive cultivation and high yields.

Corn, a staple cereal, is a cornerstone of the agricultural sector due to its versatility and adaptability. In the 2022/23 growing season, Brazil cultivated approximately 22.2 million hectares of corn, yielding 127.8 million tons (CONAB, 2023). Corn's high demand for nutrients, particularly nitrogen (N), presents a challenge in achieving optimal yields. The efficient management of N fertilization is crucial, given the crop's high N requirements and the increasing costs and environmental concerns associated with N fertilizers (Andrade et al., 2003; Coelho et al., 2004). N losses through leaching and other environmental factors can reduce crop productivity and increase costs, requiring precise fertilization strategies (Fernandes and Libardi, 2007; Hungria et al., 2007).

Soybean, another essential crop, originates from Asia and has become one of Brazil's top agricultural commodities. In the 2022/23 season, Brazil's soybean production reached 154.6 million tons, with an average yield of 3,508 kg/ha over 44.1 million hectares (CONAB, 2023). Soybean cultivation has benefitted significantly from advances in biological nitrogen fixation (BNF), a process that eliminates the need for mineral N fertilizers by utilizing diazotrophic bacteria like *Bradyrhizobium* to fix atmospheric N in root nodules (Hungria et al., 2015; Chibeba et al., 2018). This symbiotic relationship not only meets the N demands of the crop but also contributes to environmental sustainability by reducing reliance on synthetic fertilizers (Taiz and Zieger, 2013).

The success of both corn and soybean cultivation in Brazil can be attributed to ongoing research and technological advancements. One such advancement is the use of *Azospirillum brasilense*, a plant growth-promoting bacterium (PGPB) known for its ability to fix N, produce phytohormones, and enhance nutrient uptake (Cassán et al., 2020). For corn, inoculation with *A. brasilense* has shown to improve root growth, nutrient absorption, and overall plant vigor, leading to increased yields (Hungria, 2011). Similarly, in soybeans, the co-inoculation of *Bradyrhizobium* and *A. brasilense* has been widely adopted in Brazil since the introduction of strains Ab-V5 and Ab-V6 in 2013, resulting in significant gains in grain production and plant health (Hungria et al., 2013; Moretti et al., 2020).

Given the importance of both crops in Brazilian agriculture, there is a growing interest in exploring and optimizing inoculation techniques, including pre-inoculation strategies. Pre-inoculation, or the early application of inoculants, offers logistical benefits by streamlining the seed treatment process and enhancing the efficiency of planting operations (Zilli et al., 2010). While pre-inoculation has shown promising results, its effectiveness is influenced by factors such as bacterial survival, seed storage conditions, and the compatibility of inoculants with other seed treatments (Date, 2001). Therefore, further studies are necessary to fully understand the potential of this technology and its impact on the productivity of both corn and soybeans.

The objective of this study was to evaluate the viability and agronomic efficacy of the Biofix Azos inoculant (*Azospirillum brasilense* strains Ab-V5 and Ab-V6) and its co-inoculation with Biofix Protec (*Bradyrhizobium diazoefficiens* strain SEMIA 5080 and *Bradyrhizobium japonicum* strain SEMIA 5079) through a 30-day seed pre-treatment in corn and soybean, under four different edaphoclimatic regions of Brazil (the states of Paraná, São Paulo, Minas Gerais, and Goiás).

Results

The results of the concentration of *A. brasilense* and *Bradyrhizobium* cells recovered from corn and soybean seeds, both untreated and treated with Cruiser 350 FS, Maxim XL, protective additives (Protetor Ultra for corn and Protetor Protec for soybean), and Potenzial TS, inoculated on the sowing day or pre-inoculated 30 days earlier using Biofix Azos for corn or Biofix Azos and Biofix Protec for soybean, demonstrated satisfactory cell recovery for both crops (Table 1).

The recovery of cells in corn seeds inoculated with Biofix Azos and untreated with chemical products showed an average concentration on the inoculation day of 2.6×10^{10} $CFU^3.seed^{-1}$, which decreased to 2.3×10^{10} $CFU^3.seed^{-1}$ after 30 days of pre-inoculation. For seeds inoculated and pre-inoculated with Biofix Azos and treated with Cruiser 350 FS, Maxim XL, protective additives (Protetor Ultra), and Potenzial TS, the *A. brasilense* concentration was 2.5×10^{10} $CFU^3.seed^{-1}$ on the inoculation day and 2.4×10^{10} $CFU^3.seed^{-1}$ after 30 days of pre-treatment.

In soybean seeds inoculated with Biofix Azos and Biofix Protec and untreated with chemical products, the average concentration on the inoculation day was 2.1×10^{10} $CFU^3.seed^{-1}$ for *A. brasilense* and 1.2×10^{10} $CFU^3.seed^{-1}$ for *Bradyrhizobium*. After 30 days of pre-inoculation, the concentrations averaged 1.8×10^{10} $CFU^3.seed^{-1}$ and 1.9×10^{10} $CFU^3.seed^{-1}$, respectively. For seeds inoculated and pre-inoculated with Biofix Azos and Biofix Protec and treated with Cruiser 350 FS, Maxim XL, protective additives (Protetor Protec), and Potenzial TS, the concentration of *A. brasilense* was 1.8×10^{10} $CFU^3.seed^{-1}$ and *Bradyrhizobium* 1.7×10^{10} $CFU^3.seed^{-1}$ on the inoculation day, decreasing to 1.6×10^{10} $CFU^3.seed^{-1}$ and 1.2×10^{10} $CFU^3.seed^{-1}$, respectively, after 30 days of pre-treatment.

The results for the Falker chlorophyll index (FCI) and dry mass of the aerial part (DMAP) in the studied regions are presented in Table 2. In Palmeira-PR, Treatment 2 showed the highest FCI, with a value of 57.33, but there was no statistical difference compared to the other treatments. For DMAP, no significant differences were observed among treatments. In Mogi Mirim-SP, the average FCI of Treatment 4 was 63.45, significantly higher than Treatments 1 and 2 but statistically similar to Treatment 3. Regarding DMAP, all treatments were statistically similar, with averages ranging from 16.02 to 17.71 g. In Sacramento-MG, the FCI ranged from 51.73 to 56.05, with no significant differences among treatments. For DMAP, Treatments 2, 3, and 4 were statistically equal and superior to Treatment 1 (control).

The data for grain yield (GY), relative gain (RG), weight of a thousand grains (WTG), percentage of nitrogen in grains (PNG), and total nitrogen content in grains (TNCG) for corn are presented in Table 2 and organized by region. In Palmeira-PR, Treatment 4 achieved a GY of 5935.40 $kg.ha^{-1}$, with an RG of 5.5%, significantly higher than the other treatments. No significant differences were observed in WTG, which ranged from 174.62 to 179.68 g. The PNG was higher in Treatment 2, ranging from 1.46 to 1.80, while the TNCG in Treatment 4 (95.55 $kg.ha^{-1}$) was superior to Treatment 1 (control) but inferior to Treatments 2 and 3.

In Mogi Mirim-SP, Treatments 3 and 4 achieved GY values of 6696.05 and 6678.63 $kg.ha^{-1}$, respectively, without statistical differences between them but superior to other treatments. The RG was 8.0% for Treatment 3 and 7.7% for Treatment 4, both superior to Treatments 1 (control) and 2. No significant differences were observed in WTG (328.39 to 333.41 g). The PNG ranged from 1.56 to 1.78, and the TNCG in Treatment 4 was 110.87 $kg.ha^{-1}$, statistically similar to Treatment 2, superior to Treatment 1 (control), and inferior to Treatment 3. In Sacramento-MG, Treatments 3 and 4 were statistically equal to Treatment 2 for GY and WTG but superior to Treatment 1 (control). The RG was 4.9% for Treatment 3 and 2.8% for Treatment 4. The PNG was higher in Treatment 2, as also observed for the TNCG, with no significant differences among Treatments 1 (control), 3, and 4.

Table 1. Concentration of *Azospirillum* and *Bradyrhizobium* cells recovered from corn and soybean seeds with and without chemical treatment, inoculated on the day of sowing or pre-inoculated 30 days in advance with Biofix Azos and Biofix Protec.

Culture	Inoculant	Inoculation	Seed Treatment	*Azospirillum* Log 10 CFU[3].seed^{-1}	*Bradyrhizobium* Log 10 CFU[3] seed^{-1}
Corn	Biofix Azos[1]	On the day of treatment	-	$2.6.10^{10}$	-
	Biofix Azos[1]	On the day of treatment	Cruiser 350 FS + Maxim XL[4]	$2.5.10^{10}$	-
	Biofix Azos[1]	30 days after treatment	-	$2.3.10^{10}$	-
	Biofix Azos[1]	30 days after treatment	Cruiser 350 FS + Maxim XL[4]	$2.4.10^{10}$	-
Soybean	Biofix Azos[1] + Biofix Protec[2]	On the day of treatment	-	$2.1.10^{10}$	$1.2.10^{10}$
	Biofix Azos[1] + Biofix Protec[2]	On the day of treatment	Cruiser 350 FS + Maxim XL[5]	$1.8.10^{10}$	$1.7.10^{10}$
	Biofix Azos[1] + Biofix Protec[2]	30 days after treatment	-	$1.8.10^{10}$	$1.9.10^{10}$
	Biofix Azos[1] + Biofix Protec[2]	30 days after treatment	Cruiser 350 FS + Maxim XL[5]	$1.6.10^{10}$	$1.2.10^{10}$

Liquid inoculant containing *Azospirillum brasilense* strains Ab-V5 and Ab-V6. Liquid inoculant containing *Bradyrhizobium diazoefficiens* strain SEMIA 5080 and *Bradyrhizobium japonicum* strain SEMIA 5079. CFU: colony-forming unit. Added Potenzial TS and Protetor Ultra. Added Potenzial TS and Protetor Protec.

In Catalão-GO, Treatments 2, 3, and 4 presented GY values between 6060.37 and 6165.33 kg.ha^{-1}, statistically similar among them and superior to Treatment 1 (control). The RG ranged from 4.8% to 6.6%. No significant differences were observed in WTG (251.46 to 255.08 g). The PNG ranged from 1.63 to 1.88, and the TNCG among Treatments 2, 3, and 4 varied from 99.81 to 100.60 kg.ha^{-1}, with no statistical differences.

Table 3 presents the effectiveness of the treatments evaluated in terms of the number of nodules on the main root (NNMR), dry mass of nodules on the main root (DMNMR), dry mass of the aerial part (DMAP), and Falker chlorophyll index (FCI) of soybean in four regions. In Palmeira-PR, Treatment 5 was superior to Treatments 1 and 2 in NNMR and DMNMR but did not differ from Treatments 3 and 4. For DMAP, Treatment 5 reached 8.05 g, being statistically similar to Treatments 2 and 4 but different from Treatments 1 and 3. FCI ranged from 44.74 to 49.36, without significant differences among treatments.

In Itapira-SP, NNMR ranged from 11.56 to 50.56, with Treatment 5 achieving 42.20 nodules, superior to Treatments 1 (control) and 2 but inferior to Treatments 3 and 4. DMNMR in Treatment 5 was 168.08 mg, higher than Treatments 1 (control) and 2 and similar to Treatments 3 and 4. For DMAP, Treatment 5 was statistically equal to Treatments 2, 3, and 4 but superior to Treatment 1 (control). FCI ranged from 41.87 to 51.19, with Treatment 5 differing statistically from Treatments 1 (control) and 2 but not from Treatments 3 and 4. In Araguari-MG, NNMR ranged from 31.56 (Treatment 2) to 79.64 (Treatment 3), and DMNMR ranged from 111.84 to 293.00 mg, with Treatment 5 being superior to Treatments 1 and 2 but inferior to Treatments 3 and 4. Regarding DMAP, Treatments 2, 3, 4, and 5 did not differ significantly among themselves, while Treatments 2, 4, and 5 were superior to Treatment 1 (control). FCI oscillated between 42.22 and 47.46, showing no significant differences.

In Catalão-GO, Treatment 5 presented 40.73 nodules and 570.53 mg of DMNMR, differing from Treatments 1 and 2 but without statistical differences from Treatments 3 and 4. For DMAP, Treatment 5 was similar to Treatments 2, 3, and 4, and all of them were superior to Treatment 1. FCI ranged from 30.89 to 33.05, with no significant differences among treatments.

The results for grain yield (GY), relative gain (RG), weight of a thousand grains (WTG), percentage of nitrogen in grains (PNG), and total nitrogen content in grains (TNCG) in soybean cultivation are presented in Table 4.

In Palmeira-PR, Treatment 5 showed a GY of 2813.68 kg.ha^{-1}, RG of 6.0%, and TNCG of 158.69 kg.ha^{-1}. These values were statistically superior to Treatments 1 and 2 but similar to Treatments 3 and 4. For WTG, the averages ranged from 142.59 to 146.84 g, with no significant differences among treatments, and the PNG ranged from 5.51 to 5.71. In Itapira-SP, Treatment 5 achieved a GY of 3761.07 kg.ha^{-1}, RG of 6.6%, and TNCG of 199.29 kg.ha^{-1}, showing statistical similarity to Treatments 2, 3, and 4 but significantly higher than Treatment 1 (control). The WTG was 136.59 g, also statistically similar to Treatments 2, 3, and 4, but higher than Treatment 1 (control). In this region, the grain yield (PNG) ranged from 5.43 (Treatment 4) to 5.65 (Treatment 1).

In Araguari-MG, Treatment 5 presented an RG of 6.6% and a GY of 4184.90 kg.ha^{-1}, being statistically similar to Treatments 2 and 3, superior to Treatment 1 (control), but inferior to Treatment 4. For WTG, the averages ranged from 153.11 to 154.96 g, without significant differences. TNCG for Treatment 5 was statistically higher than for Treatment 1 (control) but did not differ from the other treatments. The PNG ranged from 5.64 (Treatments 3 and 4) to 5.72 (Treatment 2). In Catalão-GO, Treatment 5 showed an RG of 7.6% and a GY of 4299.94 kg.ha^{-1}, with results statistically similar to Treatments 2, 3, and 4 but significantly superior to Treatment 1 (control). In this region, the PNG ranged from 5.51 to 5.75, for WTG and TNCG, Treatment 5 showed averages statistically superior to Treatment 1 (control), with no significant differences compared to the other treatments. These findings highlight the effectiveness of Treatment 5 in promoting higher grain yield and N

Table 2. Falker chlorophyll index (FCI) and dry matter of the aerial part (DMAP) at the V6 stage, and production factors of corn: grain yield (GY), relative gain (RG), weight of a thousand grains (WTG), percentage of nitrogen in grains (PNG), and total nitrogen content in grains (TNCG) across the different evaluated regions.

PALMEIRA-PR													
Culture	Treatments	FCI[1]		DMAP (g)		GY (kg/ha)[4]		RG (%)	WTG (g)[1]		PNG %	TNCG (kg/ha)[1]	
Corn	1	48.08	b	24.55	ns	5628.07	b	0.0	174.62	ns[5]	1.46	82.17	c
	2	57.33	a	26.68	ns	5766.53	ab	2.5	176.85	ns[5]	1.80	103.77	a
	3[2]	48.23	b	26.65	ns	5865.88	ab	4.2	176.67	ns[5]	1.76	103.23	a
	4	52.87		28.60		5935.40	a	5.5	179.68	ns[5]	1.61	95.55	b
	C.V. (%)[3]	14.12	-	17.01	-	4.14	-	-	3.40	-	-	4.17	-
MOGI MIRIM-SP													
Culture	Treatments	FCI[1]		DMAP (g)[1]		GY (kg/ha)[1]		RG (%)	WTG (g)[1]		PNG %	TNCG (kg/ha)[1]	
Corn	1	59.10	c	14.51	b	6202.20	c	0.0	328.39	ns[5]	1.56	96.75	c
	2	67.42	a	17.71	a	6425.18	b	3.6	331.59	ns[5]	1.72	110.51	b
	3[2]	63.87	b	17.53	a	6696.05	a	8.0	333.41	ns[5]	1.78	119.19	a
	4	63.45	b	16.02	ab	6678.63	a	7.7	329.50	ns[5]	1.66	110.87	b
	C.V. (%)[3]	3.64	-	11.68	-	2.44	-	-	3.04	-	-	2.40	-
SACRAMENTO-MG													
Culture	Treatments	FCI		DMAP (g)[1]		GY (kg/ha)[1]		RG (%)	WTG (g)[1]		PNG %	TNCG (kg/ha)[1]	
Corn	1	51.73	ns[5]	28.38	b	8335.72	b	0.0	311.00	b	1.64	136.71	b
	2	55.78	ns[5]	30.53	a	8605.50	a	3.2	332.89	a	1.78	153.18	a
	3[2]	56.05	ns[5]	31.97	a	8742.03	a	4.9	353.86	a	1.59	139.00	b
	4	54.60	ns[5]	31.93	a	8568.40	a	2.8	339.10	a	1.63	139.66	b
	C.V. (%)[3]	7.81	-	5.51	-	1.93	-	-	5.28	-	-	1.92	-
CATALÃO-GO													
Culture	Treatments	FCI[1]		DMAP (g)[4]		GY (kg/ha)[1]		RG (%)	WTG (g)[1]		PNG %	TNCG (kg/ha)[1]	
Corn	1	53.62	b	19.53	b	5783.42	b	0.0	251.46	ns[5]	1.88	108.73	a
	2	62.37	a	22.20	a	6060.37	a	4.8	255.08	ns[5]	1.66	100.60	b
	3[2]	56.08	b	22.10	a	6165.33	a	6.6	254.69	ns[5]	1.63	100.49	b
	4	58.18	ab	22.30	a	6123.07	a	5.9	253.94	ns[5]	1.63	99.81	b
	C.V. (%)[3]	6.82	-	11.49	-	1.84	-	-	3.23	-	-	1.78	-

Means followed by the same letter in the column do not differ significantly by Duncan's test at 5% probability. Product used as the standard. Coefficient of variation in percentage. Variables analyzed at 10% probability. ns= Not significant.

Table 3. Number of nodules on the main root (NNMR), dry mass of nodules on the main root (DMNMR), dry mass of the aerial part (DMAP), Falker chlorophyll index (FCI) of soybean in the four evaluated regions.

	PALMEIRA-PR								
Culture	Treatments	NNMR[1]		DMNMR[1] mg		DMAP[1] g		FCI[1]	
Soybean	1	7.12	b	24.92	b	6.78	c	44.74	ns[5]
	2	5.12	c	14.64	c	7.23	abc	48.42	ns[5]
	3[2]	9.72	a	37.76	a	6.87	bc	47.22	ns[5]
	4[2]	8.48	a	33.96	a	7.81	ab	49.36	ns[5]
	5	9.24	a	32.60	a	8.05	a	48.90	ns[5]
	C.V. (%)[3]	12.59	-	13.37	-	9.60	-	9.29	-
	ITAPIRA-SP								
Culture	Treatments	NNMR[1]		DMNMR[1] mg		DMAP[4] g		FCI[1]	
Soybean	1	24.76	d	88.40	b	5.86	b	41.87	c
	2	11.56	e	48.40	c	8.16	a	51.19	a
	3[2]	47.04	b	176.00	a	8.22	a	48.82	b
	4[2]	50.56	a	175.60	a	8.33	a	50.84	ab
	5	42.20	c	168.08	a	7.83	a	48.91	b
	C.V. (%)[3]	6.16	-	18.81	-	18.32	-	-	-
	ARAGUARI-MG								
Culture	Treatments	NNMR[1]		DMNMR[1] mg		DMAP[1] g		FCI[1]	
Soybean	1	53.40	d	224.92	d	10.28	b	42.22	ns[5]
	2	31.56	e	111.84	e	12.20	a	47.46	ns[5]
	3[2]	79.64	a	293.00	a	11.40	ab	45.82	ns[5]
	4[2]	75.40	b	279.96	b	11.87	a	46.88	ns[5]
	5	69.92	c	267.00	c	11.64	a	46.46	ns[5]
	C.V. (%)[3]	2.01	-	1.64	-	7.39	-	9.13	-
	CATALÃO-GO								
Culture	Treatments	NNMR[1]		DMNMR[1] mg		DMAP[1] g		FCI[1]	
Soybean	1	32.20	b	486.33	b	10.21	b	30.89	ns[5]
	2	20.67	c	387.60	c	13.04	a	33.05	ns[5]
	3[2]	38.93	a	558.96	ab	12.18	a	32.16	ns[5]
	4[2]	39.33	a	564.40	a	12.26	a	32.84	ns[5]
	5	40.73	a	570.53	a	12.18	a	32.46	ns[5]
	C.V. (%)[3]	13.32	-	10.70	-	6.58	-	6.28	-

Means followed by the same letter in the column do not differ significantly by Duncan's test at 5% probability.
Product used as the standard. Coefficient of variation in percentage. Variables analyzed at 10% probability. ns= Not significant.

accumulation compared to Treatments 1 (control) and 2, while performing similarly to Treatments 3 and 4 across several analyzed variables.

The summary of the results of the analyzed variables, including the Falker chlorophyll index (FCI), dry mass of the aerial part (DMAP), for maize and soybean, and number of nodules on the main root (NNMR), and dry mass of nodules on the main root (DMNMR) for soybean are presented in Table 5. It implies that, in maize, the FCI of Treatment 4 (57.28) did not differ statistically from Treatment 3 (56.06), which was significantly higher than Treatment 1 (control) (53.13). However, it was inferior to Treatment 2, which presented the highest FCI value (60.73). The DMAP of Treatments 3 and 4 showed no significant differences between them or compared to Treatment 2. However, they were statistically higher than Treatment 1 (control). In soybean, the FCI and DMAP of Treatment 5 did not show significant differences compared to Treatments 2, 3, and 4 but exhibited statistically higher averages than Treatment 1 (control). Additionally, the NNMR and DMNMR of Treatment 5 did not differ statistically from Treatments 3 and 4 but were significantly higher than Treatments 1 (control) and 2, with averages of 40.53 nodules and 259.55 mg, respectively.

The summary of the results for grain yield (GY), relative gain (RG), thousand-grain weight (WTG), and total nitrogen content in grains (TNCG) for maize and soybean crops is presented in Table 6.

In maize, the GY across the four evaluated regions ranged from 6487.35 to 6867.33 $kg.ha^{-1}$ among treatments, with Treatments 4 and 3 standing out as statistically similar to each other but superior to Treatments 1 (control) and 2, showing RGs of 5.2% and 5.9%, respectively. For WTG, Treatments 2, 3, and 4 showed significant increases compared to Treatment 1 (control) but did not differ statistically among themselves, with averages ranging from 274.10 to 279.66 g. Regarding TNCG, Treatment 4 resulted in an average of 111.48 $kg.ha^{-1}$, with no significant differences from Treatment 3. It was significantly higher than Treatments 1 (control), but inferior to Treatment 2, which was statistically similar to Treatment 3.

In soybean, Treatment 5 presented an RG of 6.8%, with GY and TNCG differing significantly from Treatment 1 (control) but showing no statistical differences compared to the other treatments. WTG ranged from 149.38 to 153.64 g among

Table 4. Production factors of soybean: grain yield (GY), relative gain (RG), weight of a thousand grains (WTG), percentage of nitrogen in grains (PNG), and total nitrogen content in grains (TNCG) across the different evaluated regions.

PALMEIRA-PR									
Culture	Treatments	GY (kg/ha)		RG (%)	WTG (g)		PNG %	TNCG (kg/ha)	
Soybean	1	2654.42	b	0.0	142.59	ns	5.62	149.18	b
	2	2707.99	b	2.0	146.02	ns	5.67	152.46	b
	3[2]	2814.02	a	6.0	145.92	ns	5.71	160.68	a
	4[2]	2888.00	a	8.8	146.84	ns	5.51	159.13	a
	5	2813.68	a	6.0	145.31	ns	5.64	158.69	a
	C.V. (%)[3]	2.23	-	-	3.81	-	-	2.28	-
ITAPIRA-SP									
Culture	Treatments	GY (kg/ha)		RG (%)	WTG (g)		PNG %	TNCG (kg/ha)	
Soybean	1	3527.34	b	0.0	129.26	b	5.65	222.58	a
	2	3722.70	a	5.5	132.26	ab	5.48	213.89	ab
	3[2]	3792.47	a	7.5	137.37	a	5.64	209.05	bc
	4[2]	3817.88	a	8.2	137.48	a	5.43	207.23	bc
	5	3761.07	a	6.6	136.59	a	5.51	199.29	c
	C.V. (%)[3]	4.10			3.04			4.10	
ARAGUARI-MG									
Culture	Treatments	GY (kg/ha)		RG (%)	WTG (g)		PNG %	TNCG (kg/ha)	
Soybean	1	3925.99	c	0.0	153.11	ns[5]	5.65	221.82	c
	2	4175.11	b	6.3	154.22	ns[5]	5.72	236.47	ab
	3[2]	4187.94	b	6.7	154.96	ns[5]	5.64	236.20	b
	4[2]	4298.64	a	9.5	154.40	ns[5]	5.64	242.44	a
	5	4184.90	b	6.6	154.38	ns[5]	5.70	238.54	ab
	C.V. (%)[3]	1.73	-	-	3.21	-	--	1.82	-
CATALÃO-GO									
Culture	Treatments	GY (kg/ha)		RG (%)	WTG (g)		PNG %	TNCG (kg/ha)	
Soybean	1	3997.48	b	0.0	172.56	b	5.74	229.46	b
	2	4306.88	a	7.7	175.32	a	5.75	247.64	a
	3[2]	4282.82	a	7.1	176.28	a	5.75	246.28	a
	4[2]	4304.28	a	7.7	175.78	a	5.67	244.06	a
	5	4299.94	a	7.6	175.41	a	5.51	249.38	a
	C.V. (%)[3]	2.40	-	-	1.39	-	-	2.38	-

Means followed by the same letter in the column do not differ significantly by Duncan's test at 5% probability.
Product used as the standard. Coefficient of variation in percentage. Variables analyzed at 10% probability.
ns= Not significant.

treatments, with Treatment 5 showing no statistical differences from Treatments 2, 3, and 4 but being significantly superior to Treatment 1 (control).

Discussion

Azospirillum is a genus of bacteria that can associate with the rhizosphere of grasses and legumes, providing assimilable nitrogen through the biological fixation of atmospheric nitrogen (N_2) (Bulow and Von Döbereiner, 1975; Prando et al., 2019). Studies have explored the use of diazotrophic bacteria, including *Azospirillum*, due to their ability to fix nitrogen and synthesize phytohormones that promote plant growth (Montañez et al., 2009; Bashan and Bashan, 2010, Cássan et al., 2020). In Brazil, *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) has been widely used as a commercial inoculant, resulting in increased dry matter production and nitrogen accumulation in grain crops such as corn and wheat (Fukami et al., 2017; Marks et al., 2015; Hungria et al., 2010). Since 2013 the Brazilian Agricultural Research Corporation (EMBRAPA) has recommended the combined use of *A. brasilense* and *Bradyrhizobium* in seed inoculation for soybean cultivation, due to *A. brasilense* ability to stimulate root growth through phytohormone production, enhance plant nitrogen fixation efficiency, and increase productivity (Prando et al., 2019; Barbosa et al., 2021).

The practice of seed inoculation before commercialization or a few days prior to sowing (pre-inoculation) has been used for decades in various crops in other countries (Deaker et al., 2004; Herridge, 2008). In this study, satisfactory recovery of viable *A. brasilense* cells from corn seeds and *A. brasilense* and *Bradyrhizobium* cells from soybean seeds was observed, even in the presence of chemical treatments and after 30 days of seed storage. The success of pre-inoculation is inferred to result from the use of cell protectants, which ensure the survival of the bacteria for longer periods on seeds treated with insecticides and/or fungicides (Silva et al., 2018). Moreover, the biotoxic effect of agricultural chemicals on bacteria depends on the product dosage, the bacterial strains present in the inoculant, the inoculation method, and other factors (Rennie et al., 1985).

Chlorophyll is an important parameter for nitrogen absorption, as its molecule contains four nitrogen atoms at the central core, where solar radiation absorption occurs (Taiz and Zeiger, 2004). Chlorophyll content in leaves is used to predict the nitrogen nutritional status of plants because the amount of this pigment correlates positively with nitrogen content (Booij et al., 2000). This pigment is directly associated with photosynthetic potential, and the nutritional status of plants is generally related to the quantity and quality of chlorophyll (Zotarelli et al., 2003). Inoculation with *A. brasilense* bacteria frequently results in healthier, more productive plants with greater photosynthetic capacity (Bashan et al., 2004).

Table 5. Summary of evaluation results for the number of Falker chlorophyll index (FCI), dry mass of the aerial part (DMAP), nodules on the main root (NNMR), and dry mass of nodules on the main root (DMNMR), in corn and soybean crops.

Culture	Treatments	FCI[1]		DMAP (g)[1]		NNMR[1]		DMNMR[1] (mg)	
Corn	1	53.13	c	21.75	b	-	-	-	-
	2	60.73	a	24.28	a	-	-	-	-
	3[2]	56.06	b	24.56	a	-	-	-	-
	4	57.28	b	24.72	a	-	-	-	-
	C.V. (%)[3]	8.33	-	11.63	-	-	-	-	-
Soybean	1	39.93	b	8.28	b	29.38	b	206.15	b
	2	45.03	a	10.16	a	17.23	c	140.62	c
	3[2]	43.51	a	9.67	a	43.84	a	266.42	a
	4[2]	44.99	a	10.07	a	43.44	a	263.48	a
	5	44.18	a	9.93	a	40.53	a	259.55	a
	C.V. (%)[3]	7.43	-	10.32	-	22.31	-	17.81	-

Means followed by the same letter in the column do not differ significantly by Duncan's test at 5% probability.
Product used as the standard. Coefficient of variation in percentage.

Table 6. Summary of results for grain yield (GY), relative gain (RG), weight of a thousand grains (WTG), and total nitrogen content in grains (TNCG) in corn and soybean crops.

Culture	Treatments	GY (kg/ha)[1]		RG (%)	WTG (g)[1]		TNCG (kg/ha)[1]	
Corn	1	6487.35	c	0.0	266.38	b	106.09	c
	2	6714.40	b	3.5	274.10	a	117.02	a
	3[2]	6867.33	a	5.9	279.66	a	115.48	ab
	4	6826.38	a	5.2	275.55	a	111.48	b
	C.V. (%)[3]	2.59	-	-	4.59	-	6.33	-
Soybean	1	3526.31	c	0.0	149.38	b	199.94	c
	2	37286.17	b	5.7	151.95	ab	211.40	b
	3[2]	3769.32	ab	6.9	153.64	a	214.28	ab
	4[2]	3827.20	a	8.5	153.61	a	217.05	a
	5	3764.90	ab	6.8	152.92	a	213.45	ab
	C.V. (%)[3]	2.75	-	-	2.79		3.07	-

Means followed by the same letter in the column do not differ significantly by Duncan's test at 5% probability.
Product used as the standard. Coefficient of variation in percentage.

The results of the Falker chlorophyll index (FCI) in the four evaluated regions showed that Treatments 4 (corn) and 5 (soybean) did not differ significantly or were inferior to treatments which represent the use of commercial inoculants performed on the day of sowing. For Treatments 2 (corn and soybean), in soybean, the results did not show statistical differences compared to Treatment 5. However, in corn the results were superior but associated with higher production costs. These findings corroborate with those reported by Jordão et al. (2010), who observed higher total chlorophyll averages in treatments where seeds were inoculated with *A. brasilense* compared to non-inoculated treatments.

Regarding the dry matter of the aerial part (DMAP), the results showed significant increases in Treatments 4 (corn) and 5 (soybean), being superior to Treatment 1 (control) and showing no variation compared to the other treatments. Previous studies confirm these findings. Marini et al. (2015) reported that *A. brasilense* inoculation in corn crops increased dry matter mass by 11%, while Costa et al. (2015) and Brito (2019) observed increases in foliar dry mass, root volume, and nitrogen, phosphorus, and potassium contents. Nitrogen-nourished leaves have a greater capacity to assimilate CO_2 and synthesize carbohydrates during photosynthesis, resulting in greater biomass accumulation and grain yield (Ferreira, 2020). Thus, the results of this study confirm that *A. brasilense* inoculation increased chlorophyll content, aerial dry matter, and productivity in corn.

The results for grain yield (GY) demonstrate that the pre-inoculation of maize and soybean seeds with *A. brasilense* (Treatment 4 for maize and Treatment 5 for soybean) was effective in both evaluated crops, with outcomes equal to or superior to those of inoculation with *A. brasilense* on the day of sowing, and superior to Treatment 1 (control). These findings are consistent with those reported by Lana et al. (2012), who confirmed the beneficial effects of GY in maize due to inoculation with *A. brasilense*, as well as with the results observed in soybean by Hungria et al. (2013), Nogueira et al. (2018), and Prando et al. (2019). According to Hungria et al. (2013), soybean plants inoculated with *Bradyrhizobium* and *A. brasilense* exhibit double the relative gain (RG) compared to inoculation with *Bradyrhizobium* alone.

The weight of a thousand grains (WTG) is a highly relevant productive component for the evaluated crops. The results for Treatments 4 (maize) and 5 (soybean) did not show significant variations compared to Treatments 2. However, they were superior to Treatments 1 (control) in both crops, confirming the efficacy of the recommendation and use of *A. brasilense* in the pre-treatment of maize and soybean seeds. Similar results were reported by Souza (2014), who observed that the use of *A. brasilense* on maize seeds promoted greater plant height, ear insertion height, WTG, and GY. For soybean, Bárbaro et al.

(2009), when studying the co-inoculation technique (*Bradyrhizobium* and *A. brasilense*), observed higher GY and WTG with the use of inoculants based on *A. brasilense*. These findings align with Braccini (2016), who reported increases in soybean WTG with the co-inoculation of *Bradyrhizobium* and *A. brasilense*.

The variable total nitrogen content In grains (TNCG) was highly significant both for the physiological quality of the grains and for their nutritional quality, as it reflected the formation of proteins (Albrecht et al., 2008). Based on the results obtained, it was found that pre-inoculation with Biofix Azos increased TNCG in maize and soybean crops, being superior to the control and equal to or superior to other treatments, as shown in the summarized data. Furthermore, studies indicate that inoculation with *Azospirillum* increases the levels of nitrogen, phosphorus, and potassium in wheat and maize leaves, which are essential elements for plant growth and development (Galindo et al., 2016; Longhini et al., 2016).

In soybean crops, the variables number of nodules on the main root (NNMR) and dry mass of nodules on the main root (DMNMR) were also evaluated. In the studied regions, Treatment 5 showed significant differences compared to Treatments 1 and 2 but did not vary significantly compared to Treatments 3 and 4, which involve inoculation on the day of sowing with *Bradyrhizobium* and *A. brasilense*, or *A. brasilense* alone. Thus, it was observed that the use of Biofix Azos and Biofix Protec was effective in the pre-inoculation of soybean seeds for 30 days.

The literature indicates that the success of nodulation depends on the interaction between *Bradyrhizobium*, *A. brasilense* and the plant, mediated by compounds synthesized by roots, primarily flavonoids, which attract rhizobia to the rhizosphere and activate *nod* genes responsible for nodule primordium formation (Cai et al., 2009; Carvalhais et al., 2013; Timmers et al., 1999). Studies suggest that *A. brasilense* can induce the synthesis of flavonoids, increasing the attractiveness of rhizobia to the rhizosphere (Dardanelli et al., 2008). These findings corroborate to the results of this study, where pre-co-inoculation with Biofix Azos (*A. brasilense*) and Biofix Protec (*B. diazoefficiens* and *B. japonicum*), performed 30 days prior to sowing, improving NNMR, DMNMR, and GY in soybean.

Research on seed pre-inoculation has been receiving growing prominence in recent years, as this practice offers important advantages to farmers such as optimizing the sowing window, improving logistical efficiency, and enhancing the practicality of the inoculation process (Hungria et al., 2017). The development of longer shelf-life inoculants is crucial to support this strategy, as it enables seed treatment to be performed in advance while maintaining the viability of the microorganisms. Furthermore, this technological advancement incurs no substantial increase in production costs, making it a viable and efficient alternative for large-scale agricultural operations (Schweig et al., 2017; Hungria et al., 2017).

Considering the promising and effective results of the 30-day pre-inoculation with Biofix Azos in maize and Biofix Azos and Biofix Protec in soybean, this study recommends the use of these inoculants due to the operational ease of early seed treatment and the promotion of growth and productivity in these crops.

Materials and Methods

Laboratory trial

The laboratory trials evaluated the survival of bacterial cells in corn and soybean seeds treated with Biofix Azos, Biofix Protec (for soybean), chemical treatment, and protective additives (Table 7). The seeds were treated immediately before being sent to the Nema Brasil Laboratory to conduct cell survival analysis.

Each experimental unit consisted of 1 kg of seeds, which were stored in double paper bags inside a B.O.D. incubator under controlled temperatures: 18-20 °C for corn and 15-18 °C for soybean. Bacterial survival was assessed at two intervals: on the day of treatment (Day 0) and 30 days after treatment (pre-sowing).

From each experimental unit, 100 seeds were sampled for bacterial survival analysis. The analysis of *Azospirillum brasilense* for corn and *A. brasilense* and *Bradyrhizobium* for soybean followed the official methodology recommended by the Ministry of Agriculture, Livestock, and Food Supply (MAPA) as outlined in Normative Instruction No. 30 of November 17, 2010. Additionally, the soybean trial incorporated analysis based on the protocol included in MAPA's Public Consultation Portaria 341 (D.O.U. 01/10/2009).

Product description and doses

In the corn crop, N fertilization in treatments with 100% N consisted of applying urea (46-00-00) in sufficient quantity to meet the recommendation of 200 $kg.ha^{-1}$ of N, with 30% applied at sowing and 70% as top-dressing when the crop was at the V4 phenological stage. The standard inoculation involved the application of the commercial inoculant, formulated with the bacteria *Azospirillum brasilense* Ab-V5 and Ab-V6, following the label's technical recommendation. The tested inoculant, Biofix Azos, is a liquid formulation containing *Azospirillum brasilense* Ab-V5 and Ab-V6 at a final concentration of $2x10^8$ $CFU.mL^{-1}$.

In the soybean crop, N fertilization was performed in the Treatment 2, with the application of urea (46-00-00) in a quantity sufficient to meet the recommendation of 200 $kg.ha^{-1}$ of N, applied at two stages: 50% at planting and 50% at the R1 stage. The standard co-inoculation involved the application of the commercial inoculant, formulated with *Azospirillum brasilense* Ab-V5 and Ab-V6, along with commercial inoculant containing *Bradyrhizobium japonicum* (SEMIA 5079 and SEMIA 5080), following the label's technical recommendation. The tested inoculant, Biofix Azos, is a liquid formulation containing *Azospirillum brasilense* Ab-V5 and Ab-V6 at a final concentration of $2x10^8$ $CFU.mL^{-1}$, co-inoculated with Biofix Protec, a liquid formulation composed of *Bradyrhizobium diazoefficiens* – SEMIA 5080 and *Bradyrhizobium japonicum* – SEMIA 5079, at a final concentration of $5x10^9$ $CFU.mL^{-1}$.

Table 7. Description of treatments used in the laboratory trial for viable cell recovery.

Culture	Treatment	Chemical treatment	Additive protectors
Corn	Biofix Azos 0 day	-	No
	Biofix Azos 30 days	-	No
	Biofix Azos 0 day	Cruiser 350 FS + Maxim XL	Yes
	Biofix Azos 30 days	Cruiser 350 FS + Maxim XL	Yes
Soybean	Biofix Azos + Biofix Protec 0 day	-	No
	Biofix Azos + Biofix Protec 30 days	-	No
	Biofix Azos + Biofix Protec 0 day	Cruiser 350 FS + Maxim XL	Yes
	Biofix Azos + Biofix Protec 30 days	Cruiser 350 FS + Maxim XL	Yes

Table 8. Description of the four edaphoclimatic regions where the tests were conducted.

Culture	Region	Municipality	Latitude	Longitude
Corn	South	Palmeira - PR	25°25’28.92’’ S	50°03’04.24’’ O
	Southeast	Mogi Mirim - SP	22°28’55.91’’ S	47°01’26.89’’ O
	Midwest	Sacramento - MG	19°38’49.50’’ S	47°31’00.10’’ O
	Midwest	Catalão - GO	18°05’18.46’’ S	47°52’34.05’’ O
Soybean	South	Palmeira - PR	25º25’26.44’’ S	50º03’07.41 ’’O
	Southeast	Itapira - SP	22°23’51.73 ’’S	46°46’10.73 ’’O
	Midwest	Araguari - MG	18°31’50.89 ’’S	48°02’59.24 ’’O
	Midwest	Catalão - GO	18°05’41.81 ’’S	47°58’35.02 ’’O

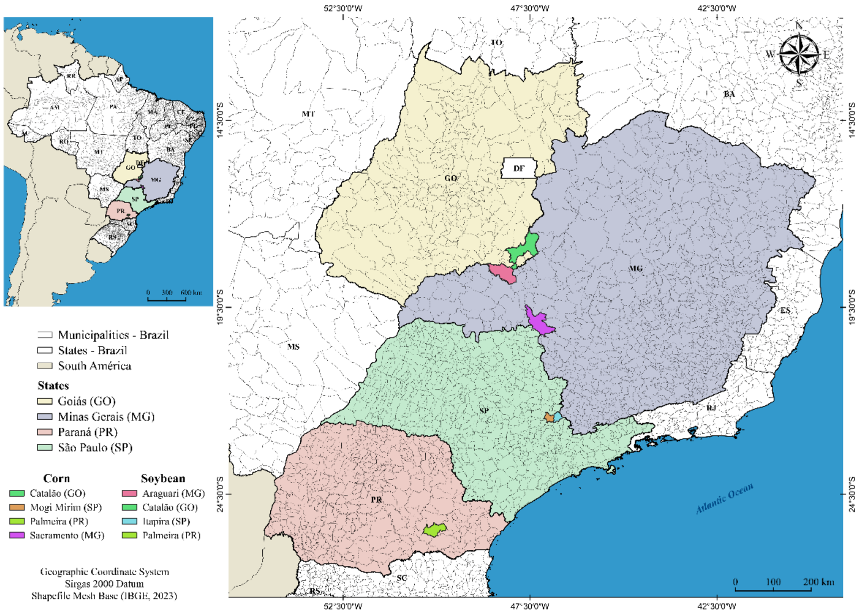

Figure 1. Distribution of testing sites.

Treatments description

Corn treatments

Treatment 1: Control (no inoculation, and no N application);

Treatment 2: Fertilization with 200 $kg.ha^{-1}$ of N, 30% applied at planting and 70% as top-dressing;

Treatment 3: Commercial seed inoculant, formulated with the bacteria *Azospirillum brasilense* strains Ab-V5 and Ab-V6, at a concentration of 2×10^8 CFU mL^{-1} (100 mL/50 kg of seeds), following the technical recommendation on the label, applied on the sowing day. The application of N in the treatments with 100% N was carried out as follows: 30% at the time of sowing and 70% as top-dressing;

Treatment 4: Seed inoculation with Biofix Azos (100 mL/50 kg of seeds), formulated with the bacteria *Azospirillum brasilense* strains Ab-V5 and Ab-V6, at a concentration of 2×10^8 CFU mL^{-1}, Biofix Protec (100 mL/50 kg of seeds), combined with the following products: cell protector Protetor Ultra (0.5 mL/kg of seeds), and Potenzial TS (1.25 mL/kg of seeds), applied 30 days before planting, and the N application for the treatments with 100% N was divided into two stages: 30% at sowing and 70% as top-dressing.

Soybean treatments

Treatment 1: Control (no inoculation, and no N application);

Treatment 2: Fertilization with 200 $kg.ha^{-1}$ of N, 50% applied at planting and 50% applied at flowering;

Treatment 3: Commercial seed inoculant (100 mL/50 kg of seeds), formulated with the bacteria *Bradyrhizobium japonicum* strains SEMIA 5079 and 5080, at concentration of $5x10^9$ CFU mL^{-1}, following the technical recommendation on the label, applied on the sowing day;

Treatment 4: Seed inoculation with two commercial inoculants. The first inoculant was formulated with the bacteria *Azospirillum brasilense* strains Ab-V5 and Ab-V6, at a concentration of 2×10^8 CFU mL^{-1} (100 mL/50 kg of seeds). The second inoculant was formulated with the bacteria *Bradyrhizobium japonicum* strains SEMIA 5079 and 5080, at a concentration of 5×10^9 CFU mL^{-1} (100 mL/50 kg of seeds). Following the technical recommendations provided on the product labels, applied on the sowing day.

Treatment 5: Seed inoculation with Biofix Azos (100 mL/50 kg of seeds), formulated with the bacteria *Azospirillum brasilense* strains Ab-V5 and Ab-V6, at a concentration of 2×10^8 CFU mL^{-1}, Biofix Protec (100 mL/50 kg of seeds), a liquid inoculant made of the bacteria *Bradyrhizobium japonicum* –SEMIA 5079 and *Bradyrhizobium diazoefficiens* –SEMIA 5080, at a final concentration of $5x10^9$ CFU mL^{-1}, combined with the following products: cell protector Protetor Protec (0.5 mL/kg of seeds), and Potenzial TS (0.2 mL/kg of seeds), applied 30 days before planting.

Field test methodology

In the 2022-2023 agricultural season, trials were conducted in four municipalities representing corn and soybean cultivation across Brazil's central-west, southeast, and southern regions, each with distinct edaphic and climatic characteristics (Table 8). This multi-regional approach allows for the evaluation of treatment efficacy under diverse soil and climate conditions, enhancing the reliability and applicability of the results across different agricultural contexts.

Corn trials involved four treatments with six replications. In treatments receiving 100% N, 200 $kg.ha^{-1}$ of N was applied, 30% at sowing and 70% at the V4 stage. In Catalão (GO) and Sacramento (MG), N was partially applied as formulated fertilizer, supplemented by urea. Soybean trials, featuring five treatments and five replications, included 200 $kg.ha^{-1}$ N split between sowing and the R1 stage. Urea (46-00-00) was applied at a rate of 435 $kg.ha^{-1}$.

For each treatment, 4 kg of soybean and corn seeds were treated using an industrial seed treatment machine. Fungicides and insecticides were applied sequentially to the seeds in all treatments (fungicide: Maxin XL - Metalaxil-M $10g.L^{-1}$ + Fludioxonil $25g.L^{-1}$, and insecticide: Cruiser 350 FS - Tiametoxam 350 $g.L^{-1}$). Seeds were pre-treated for 30 days before planting to follow the schedule for each region in the experiment.

For both soybean and corn, the solution volume did not exceed 300 mL per 50 kg of seeds. After treatment and complete drying, the seeds were stored in paper containers, in a dry, dark place with temperatures between 18-20 °C and humidity levels below 70%.

The experimental design for the corn crop followed a randomized block layout with four treatments and six replications. Each plot measured 4.0 meters in width and 6.0 meters in length, totaling 24.0 m^2. The GY was harvested from the four central rows of each plot, excluding 1 meter from each end. Similarly, in the soybean crop, a randomized block design was employed with five treatments and five replications. Each plot also measured 4.0 meters in width and 6.0 meters in length, totaling 24.0 m^2. The harvest area comprised of four central rows with 0.5 meters excluded from each end, resulting in a useful area of 6.0 m^2.

Variables were measured following official agronomic efficiency protocols (Brazil, 2011). The dry shoot biomass was analyzed in both corn and soybean crops using a standardized methodology. For corn, assessments were conducted at the V6 phenological stage, with five plants sampled per plot. Drying was performed in a forced-air oven at 65°C until a constant weight was achieved, and the data were expressed as grams per plant ($g.plant^{-1}$). Similarly, in the soybean crop, five plants were sampled and dried under identical conditions, with the results also reported in grams per plant.

The N content in leaves were determined, with N measured via chlorophyll content using the ClorofiLOG® device. GY and 1000-grain weight were adjusted to 13% moisture, and N content in grains was analyzed using AOAC (2019) method. Data were analyzed using ANOVA and Duncan's test for significant results at 1%, 5%, and 10% levels.

Conclusion

The pre-inoculation of Biofix Azos (for maize) and Biofix Azos and Biofix Protec (for soybean) up to 30 days before sowing is effective for both soybean and maize crops. In maize, Biofix Azos promoted an increase in the dry matter of the aerial part, contributed to chlorophyll content, enhanced grain yield and nitrogen content, and resulted in yield gains. In soybean, this approach improved nodulation, the dry mass of nodules and shoots, grain productivity, nitrogen content, and other physiological parameters such as chlorophyll content. The results were statistically similar to those obtained with inoculation performed on the sowing day, confirming the technical feasibility of pre-treatment, even when combined with chemical seed treatments. Moreover, the use of cell protector ensured the survival of *A. brasilense* and *Bradyrhizobium* cells, emphasizing the sustainability of this practice. Pre-inoculation provides direct benefits to the producers by optimizing processes and contributing to more efficient agriculture aligned with economic, social, and environmental sustainability demands.

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