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Agronomic Efficiency of Grammy Crop® (*Azospirillum brasilense*) Under Different Application Methods in First- and Second-Crop Maize

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Abstract

The nutritional requirements of maize are some of the most studied factors in the production of this crop. It is known that nitrogen (N) is the major nutrient required by maize. Products based on microorganisms such as *Azospirillum* spp. can promote N fixation, providing yield gains. This study aimed to investigate the agronomic efficiency and feasibility of applying Grammy Crop® (*Azospirillum brasilense*-based product) by different methods to first- and second-crop maize. Experiments were conducted in two seasons between 2019 and 2020. In the first crop, a 4 × 7 factorial design was used, in which the first factor was locality (Candói, PR; Guarapuava, PR; Santa Maria do Oeste, PR; and Sertão, RS) and the second factor was treatment (untreated control, 75% N control, 100% N control, commercial *A. brasilense*-based inoculant, Grammy Crop® liquid seed treatment, Grammy Crop® peat-based seed treatment, and Grammy Crop® liquid foliar treatment). In the second crop, the factorial design was 3 × 7, with three localities (Pitanga, PR; São Miguel do Iguaçu, PR; and Serranópolis do Iguaçu, PR) and the same treatments. At physiological maturity, plants were evaluated for yield (kg ha^{-1}), thousand grain weight (g), plant height (cm), shoot dry weight (kg ha^{-1}), leaf N content (g kg^{-1}), and grain N content (g kg^{-1}). All treatments improved productive, vegetative, and nutritional parameters compared with the untreated control for all localities and crops. Grammy Crop® was efficient in improving maize yield under all methods of application, providing similar benefits as N fertilizer and a commercial inoculant.

Keywords: inoculant, nitrogen fixation, plant growth-promoting bacteria, yield, *Zea mays*

1. Introduction

Maize (*Zea mays*) is one of the most cultivated cereals worldwide, having high economic relevance because of the nutritional value of its grains, which are used as human and animal food and as raw materials in different industries (Kamran et al., 2020; Yang & Yan, 2021). The growing global demand for maize and its derivatives has motivated research on strategies to enhance crop yield (Hussain et al., 2022).

Nutritional requirements are some of the most studied factors in maize cultivation. Nitrogen (N) is the nutrient required in greater amounts by maize crops, being therefore directly related to grain yield (Wani et al., 2021). N is an essential component of plant amino acids, proteins, and enzymes. N-deficient plants show characteristic signs of yellowing in older leaves, followed by generalized chlorosis and leaf loss (Marion et al., 2021).

The high economic and environmental costs of industrial N fixation, combined with the predicted increase in food demand, underscores the need for novel agricultural technologies aimed at rationalizing the use of synthetic N fertilizers (Mrid et al., 2021). Microbial products represent an emerging technology designed to improve agricultural yields in the long term. Such products are aligned with the principles of sustainable agriculture in the fight against the widespread use of large amounts of pesticides and fertilizers (Lozowicka et al., 2021). Plant growth-promoting bacteria (PGPB) are prominent examples of microbial agents with great potential in sustainable agriculture. These beneficial microorganisms colonize plant roots and increase crop yield and productivity, constituting an efficient alternative to chemical fertilizers, pesticides, and supplements (Toribio-Jimenez et al., 2017).

Azospirillum brasilense is the PGPB that provides the best results for maize crops. This N-fixing microorganism colonizes plant roots and produces growth-promoting substances, such as indoleacetic acid, gibberellins, pantothenic acid, thiamine, and niacin, increasing root density and branching (Housh et al., 2021). Inoculation of maize with *A. brasilense* was shown to improve root development, water and nutrient absorption, and tolerance to stress conditions, such as high salinity and water deficit (Housh et al., 2021; Marques et al., 2021). Given its beneficial effects, this bacterium holds potential as a biological agent to increase crop yield and reduce losses by stimulating the use efficiency of nutrients available in the plant environment (Galindo et al., 2020).

In view of the above, this study aimed to assess the agronomic efficiency and feasibility of using Grammy Crop® (a product based on *A. brasilense*) in maize crops under different methods of application.

2. Material and Methods

2.1 General Experimental Procedures

The study was conducted in the field, with four trials for the first crop (2019/2020) and three trials for the second crop (2019/2020), under different edaphoclimatic conditions. The localities chosen for field tests of the first crop were Candói, Guarapuava, and Santa Maria do Oeste, Paraná State, and Sertão, Rio Grande do Sul State, Brazil. Second-crop maize was planted in Pitanga, São Miguel do Iguaçu, and Serranópolis do Iguaçu, Paraná State (Table 1).

Table 1. Climatic data of each locality during the conduction of the experiments

Locality	Temperature (°C)			Precipitation (mm)
	Minimum	Maximum	Average	
Candói	16.6	26.1	21.3	1093
Guarapuava	15.1	24.3	19.7	1190
Santa Maria do Oeste	15.6	24.6	20.1	1212
Sertão	16.0	25.9	20.9	1271
Pitanga	14.2	23.7	18.9	1926
São Miguel do Iguaçu	18.0	26.9	22.5	1800
Serranópolis do Iguaçu	16.9	28.9	22.9	2124

Each experimental unit consisted of eight 6.0 m long rows spaced 0.50 m apart. In the first season, maize DKB 345 PRO3 (early cycle) was grown at a density of 70 000 plants ha^{-1}. In the second season, maize DKB 265 PRO3 (super early cycle) was grown at a density of 60 000 plants ha^{-1}.

2.2 Treatments

At all sites, experiments were conducted using a randomized block design with six replications. A 4 × 7 (Locality × Treatment) factorial arrangement was used in the first season and a 3 × 7 (Locality × Treatment) factorial arrangement in the second season. Treatments were the same in all experiments, as follows: untreated control (without product application), 75% N control (N rate of 75% applied by top dressing, where a rate of 100% corresponds to 200 kg ha^{-1}), 100% N control (N rate of 100% applied by top dressing), commercial inoculant (Nitro 100® Gramíneas, liquid commercial product containing *A. brasilense* Ab-V5 (= CNPSo 2083) and Ab-V6 (= CNPSo 2084) at 2.0×10^8 CFU mL^{-1}, applied via seed treatment at a rate of 100 mL per 60 000 or 70 000 seeds), Grammy Crop® liquid seed treatment (*A. brasilense* Ab-V5 and Ab-V6 at 2.0×10^8 CFU mL^{-1}, applied via seed treatment at a rate of 100 mL per 60 000 or 70 000 seeds), Grammy Crop® liquid foliar treatment (*A. brasilense* Ab-V5 and Ab-V6 at 2.0×10^8 CFU mL^{-1}, applied at a rate of 300 mL ha^{-1} by spraying the leaves of plants at the V3 stage in the first crop and V4 stage in the second crop), and Grammy Crop® peat-based seed treatment (*A. brasilense* Ab-V5 and Ab-V6 at 2.0×10^8 CFU mL^{-1}, applied via seed treatment at a rate of 100 g per 60 000 or 70 000 seeds).

Application of Grammy Crop® via seed treatment was performed in a shaded area, immediately before sowing. Foliar application was performed when the fourth leaf was completely expanded. Application at phenological stage V3 or V4 was performed using a battery-powered backpack sprayer at a rate of 160 L ha^{-1}. Application was carried out from 16:00 h onward to avoid high temperatures.

2.3 Yield Parameters

When the crop reached physiological maturity, the three center rows of the plot were harvested, discarding 0.50 m from each edge as borders. The material was threshed and dried. Grain yield (kg ha^{-1}) was calculated and adjusted to 13% moisture using the following equation (Equation 1):

$$Y = \frac{W_f\,(100 - M)}{87} \tag{1}$$

where, Y is the yield adjusted to 13% moisture, W_f is the fresh weight, and M is the grain moisture.

From a subsample of the harvested material, 300 grains from each plot were counted and weighed for determination of thousand grain weight, which was calculated as follows (Equation 2):

$$\text{Thousand grain weight} = \frac{W \times 1000}{300} \tag{2}$$

where, W is the weight of 300 grains.

2.4 Vegetative Parameters

Plant height (cm) was determined in three randomly chosen plants from the useful area of plots by direct measurement using a graduated ruler. Height was measured from ground level up to the flag leaf.

Five plants at R1 were harvested from the useful area of each plot. Shoot fresh weight was determined by weighing plants. Then, the material was ground, and aliquots of 150 g were dried in a forced-air oven at 65 °C for 72 h to determine shoot dry weight (kg ha^{-1}).

2.5 N content in Shoots and Grains

Leaf N content (g kg^{-1}) was determined in subsamples of the material used for shoot weight determination. Grains used for N content (g kg^{-1}) determination were obtained at harvest. Shoot and grain samples were dried in a forced air oven at 65 °C until constant weight was achieved. Subsequently, samples were ground in a Wiley-type mill equipped with a 1 mm diameter sieve and sent to the Soil and Plant Nutrition Laboratory at UNICENTRO to determine leaf and grain N contents. Analyses were conducted according to the method proposed by the EMBRAPA Manual of Chemical Analysis of Soils, Plants, and Fertilizers (Silva, 2009).

2.6 Statistical Analysis

Data were tested for normality of distribution and homogeneity of variances by Shapiro-Wilk and Levene's tests, respectively. Two-way ANOVA was used to evaluate the effect of locality, treatment, and their interactions. For assessing the main effects of locality, treatment means were subjected to Tukey's test ($p < 0.05$). The Scott-Knott test was used to assess the main effects of treatments ($p < 0.05$). All analyses were conducted using Sisvar software version 5.6 (Ferreira, 2014).

3. Results

In the first crop, there were significant interaction effects on yield, thousand grain weight, and leaf N content (Table 2). For plant height, shoot dry weight, and grain N content, interaction effects were not significant but the main effects of treatment and locality were (Table 2).

Table 2. Analysis of variance (mean squares) for yield, thousand grain weight (TGW), plant height, shoot dry weight, grain nitrogen (N) content, and leaf N content in first-crop maize

Source of variation	df	Mean squares					
		Yield	TGW	Plant height	Shoot dry weight	Grain N	Leaf N
Blocks	5	732 538.26 ns	468.29 ns	27.65 ns	1 004 533.32 ns	1.23 ns	2.84 ns
Locality (*L*)	2	208 250 790.71 *	125 297.65 *	14 715.74 *	151 648 420.29 *	31.66 **	196.19 **
Treatment (*T*)	6	74 947 586.66 *	7591.43 *	523.48 *	26 314 894.97 *	41.98 **	483.23 **
L × *T*	12	4 426 184.55 *	690.37 *	115.38 ns	921 812.57 ns	2.52 ns	11.74 **
Error	100	592 021.65	255.74	153.61	2 147 900.46	1.56	4.97
Mean	-	12 485.82	412.71	253.42	12 811.99	12.63	26.67

Note. df, degrees of freedom; * significant at $p < 0.05$; ** significant at $p < 0.01$; ns, not significant.

In the second crop, the interaction of factors did not influence plant height, shoot dry weight, or grain and leaf N content. Locality had a significant effect on thousand grain weight (Table 3).

Table 3. Analysis of variance (mean squares) for yield, thousand grain weight (TGW), plant height, shoot dry weight, grain nitrogen (N) content, and leaf N content in second-crop maize

Source of variation	df	Mean squares					
		Yield	TGW	Plant height	Shoot dry weight	Grain N	Leaf N
Blocks	5	1 103 363 *	884.17 ns	217.69 *	1 968 743 ns	2.89 ns	20.64 *
Locality (*L*)	2	86 643 795 *	45 915.37 *	25 701.81 *	338 668 300 *	19.25 *	1312.51 *
Treatment (*T*)	6	5 094 172 *	1188.31 *	53.70 ns	3 170 145 *	6.85 *	36.08 *
$L \times T$	12	521 837 ns	260.17 ns	47.12 ns	826 830 ns	5.06 *	13.15 *
Error	100	344 938.18	392.59	54.49	1 076 715	1.98	6.71
Mean	-	8621.72	354.09	201.74	9545.23	14.98	28.45

Note. df, degrees of freedom; * significant ($p < 0.05$); ns, not significant.

In both crops, all treatments increased maize yield compared with the untreated control in all localities (Table 4). In the first crop, Guarapuava and Sertão had the highest and lowest yields, respectively, and Candói and Santa Maria do Oeste had intermediate yields. In the second crop, the yield was highest in Pitanga, lowest in Serranópolis do Iguaçu, and intermediate in São Miguel do Iguaçu (Table 4).

Table 4. Yield (kg ha^{-1}) and thousand grain weight (g) of first-crop maize (2019/2020) grown in Candói (PR), Guarapuava (PR), Santa Maria do Oeste (PR), and Sertão (RS) and second-crop maize (2019/2020) grown in Pitanga (PR), São Miguel do Iguaçu (PR), and Serranópolis do Iguaçu (PR)

Treatment	Yield (kg ha^{-1})							
	First crop				Second crop			
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu	$\bar{x}$
Grammy Crop® liquid foliar treatment	14 225 a	15 581 a	14 189 a	10 020 a	9864	9636	7490	8997 a
Grammy Crop® peat-based seed treatment	14 136 a	15 409 a	14 135 a	9762 a	9739	9644	7323	8902 a
Grammy Crop® liquid seed treatment	13 932 a	15 159 a	14 106 a	9737 a	9885	9677	7079	8880 a
Commercial inoculant	13 837 a	15 126 a	13 938 a	9498 a	9857	9390	6829	8692 b
100% N control	13 817 a	14 973 a	13 913 a	9400 a	9902	9638	7393	8978 a
75% N control	13 288 a	14 860 a	13 174 a	9322 a	9443	9100	6623	8389 b
Untreated control	11 194 b	7915 b	8231 b	6726 b	8976	7417	6151	7514 c
$\bar{x}$	13 490 B	14 146 A	13 098 B	9209 C	9666 A	9215 B	6984 C	
CV (%)	6.2				7.7			

Treatment	Thousand grain weight (g)							
	First crop				Second crop			
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu	$\bar{x}$
Grammy Crop® liquid foliar treatment	454 a	439 a	444 a	343 a	370.6	388.6	321.3	360 a
Grammy Crop® peat-based seed treatment	473 a	440 a	439 a	333 a	369.3	383.2	319.2	357 a
Grammy Crop® liquid seed treatment	459 a	442 a	447 a	334 a	370.1	385.2	317.8	357 a
Commercial inoculant	444 b	436 a	440 a	330 a	375.0	378.3	310.2	354 a
100% N control	463 a	448 a	451 a	337 a	374.2	378.9	323.8	359 a
75% N control	460 a	427 a	440 a	342 a	371.8	369.9	319.2	354 a
Untreated control	434 b	365 b	384 b	309 b	362.2	346.4	300.7	336 b
$\bar{x}$	455 A	428 B	435 B	333 C	370 A	376 A	318 B	
CV (%)	3.9				5.8			

Note. Means followed by the same lowercase letter are not significantly different from each other by the Scott-Knott test ($p < 0.05$), and means followed by the same uppercase letter are not significantly different from each other by Tukey's test ($p < 0.05$). CV, coefficient of variation.

All treatments increased plant height and shoot dry weight in both crops (Table 5). In the first crop, the lowest plant heights were observed in Sertão. In the second crop, plant height was highest in Pitanga, lowest in Serranópolis do Iguaçu, and intermediate in São Miguel do Iguaçu (Table 5). In the first crop, Candói and Guarapuava had the highest shoot dry weights, followed by Santa Maria do Oeste and Sertão. In the second crop, the results of shoot dry weight followed the same pattern as for plant height (Table 5).

Table 5. Plant height (cm) and shoot dry weight (kg ha^{-1}) of first-crop maize (2019/2020) grown in Candói (PR), Guarapuava (PR), Santa Maria do Oeste (PR), and Sertão (RS) and second-crop maize (2019/2020) grown in Pitanga (PR), São Miguel do Iguaçu (PR), and Serranópolis do Iguaçu (PR)

Treatment	Plant height (cm)							
	First crop					Second crop		
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	$\bar{x}$	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu
Grammy Crop® liquid foliar treatment	267	262	262	228	254 a	228	200	183
Grammy Crop® peat-based seed treatment	265	262	267	223	254 a	230	200	181
Grammy Crop® liquid seed treatment	267	261	266	223	254 a	226	202	177
Commercial inoculant	264	265	266	227	256 a	226	202	172
100% N control	264	265	269	224	256 a	228	195	174
75% N control	265	264	266	232	256 a	229	198	180
Untreated control	260	250	240	221	243 b	225	202	178
$\bar{x}$	264 A	262 A	262 A	225 B		227 A	200 B	178 C
CV (%)	4.8					3.9		

Treatment	Shoot dry weight (kg ha^{-1})								
	First crop					Second crop			
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	$\bar{x}$	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu	$\bar{x}$
Grammy Crop® liquid foliar treatment	14 699	15 074	13 954	10 501	13 557 a	12 179	9008	7420	9869 a
Grammy Crop® peat-based seed treatment	14 517	15 523	13 610	10 548	13 401 a	13 451	8697	7244	9797 a
Grammy Crop® liquid seed treatment	14 806	14 151	13 922	10 726	13 550 a	13 275	8979	7258	9837 a
Commercial inoculant	14 715	13 768	13 268	10 712	13 116 a	12 979	8755	7465	9733 a
100% N control	12 055	11 803	10 373	8337	13 103 a	12 795	8523	7303	9540 a
75% N control	14 602	14 088	13 657	10 064	12 316 b	12 284	8552	7209	9348 a
Untreated control	13 493	13 416	12 940	9415	10 642 c	11 056	8241	6778	8691 b
$\bar{x}$	14 127 A	13 975 A	13 104 B	10 043 C		12 717 A	8679 B	7240 C	
CV (%)	11.3					11.8			

Note. Means followed by the same lowercase letter are not significantly different from each other by the Scott-Knott test ($p < 0.05$), and means followed by the same uppercase letter are not significantly different from each other by Tukey's test ($p < 0.05$). CV, coefficient of variation.

In the first crop, grain N content was increased by all treatments, whereas, in the second crop, only the 75% N control treatment afforded an increase in the parameter (Table 6). Higher shoot dry weights were obtained in Santa Maria do Oeste in the first crop and Pitanga and Serranópolis do Iguaçu in the second crop (Table 6). All treatments increased leaf N content, with higher means observed in Sertão in the first crop and Pitanga in the second crop (Table 6).

Table 6. Grain and leaf nitrogen (N) contents (g kg^{-1}) in first-crop maize (2019/2020) grown in Candói (PR), Guarapuava (PR), Santa Maria do Oeste (PR), and Sertão (RS) and second-crop maize (2019/2020) grown in Pitanga (PR), São Miguel do Iguaçu (PR), and Serranópolis do Iguaçu (PR)

Treatment	Grain N content (g kg^{-1})							
	First crop					Second crop		
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	$\bar{x}$	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu
Grammy Crop® liquid foliar treatment	12.4	12.7	14.0	13.4	13.1 a	15.1^{ns}	14.7 a	15.0 b
Grammy Crop® peat-based seed treatment	12.6	12.9	13.7	13.4	13.2 a	16.6	14.8 a	14.6 b
Grammy Crop® liquid seed treatment	12.3	12.4	15.4	13.1	13.3 a	15.2	14.9 a	15.4 b
Commercial inoculant	11.4	12.9	13.8	13.4	12.9 a	16.4	14.6 a	14.5 b
100% N control	11.6	12.5	13.8	13.7	12.9 a	15.6	14.4 b	15.0 b
75% N control	11.7	13.0	15,.1	13.9	13.4 a	14.9	14.9 a	17.2 a
Untreated control	10.3	9.0	10.6	8.8	9.7 b	15.6	11.9 a	13.7 b
$\bar{x}$	11.8 C	12.2 CB	13.8 A	12.8 B		15.6 A	14.3 B	15.1 A
CV (%)	9.8					9.5		

Treatment	Leaf N content (g kg^{-1})							
	First crop				Second crop			
	Candói	Guarapuava	Santa Maria do Oeste	Sertão	Pitanga	São Miguel do Iguaçu	Serranópolis do Iguaçu	$\bar{x}$
Grammy Crop® liquid foliar treatment	26.2 a	26.8 a	28.0 a	31.8 b	34.6	23.3	29.4	29.1 a
Grammy Crop® peat-based seed treatment	26.0 a	27.7 a	26.7 a	34.0 a	34.0	23.3	29.1	28.8 a
Grammy Crop® liquid seed treatment	26.4 a	27.8 a	27.7 a	30.6 b	34.0	23.2	28.1	28.4 a
Commercial inoculant	26.1 a	27.7 a	28.0 a	30.3 b	34.6	22.5	30.1	29.0 a
100% N control	26.7 a	27.8 a	28.9 a	32.7 a	32.8	25.1	28.8	28.9 a
75% N control	26.0 a	26.3 a	28.0 a	33.3 a	33.2	23.1	32.5	29.6 a
Untreated control	18.1 b	15.1 b	16.2 b	16.7 c	32.2	17.1	26.7	25.3 b
$\bar{x}$	25.1 B	25.6 B	26.2 B	29.8 A	33.6 A	22.5 C	29.2 B	
CV (%)	8.3				9.5			

Note. Means followed by the same lowercase letter are not significantly different from each other by the Scott-Knott test ($p < 0.05$), and means followed by the same uppercase letter are not significantly different from each other by Tukey's test ($p < 0.05$). CV, coefficient of variation.

4. Discussion

Inoculation of maize seeds with Grammy Crop® (*A. brasilense*) was efficient in improving crop development and yield by all application methods. In Brazil, the beneficial effect of *A. brasilense* inoculation in maize is well documented. This treatment significantly increases grain yield and N accumulation in plants, being a viable and sustainable alternative for ensuring N supply (Oliveira et al., 2018; Bertaselli et al., 2021).

The beneficial effects of *A. brasilense* and other PGPB are related to the ability of these bacteria to stimulate plant growth through processes such as biological N fixation, synthesis of plant hormones and other important molecules for metabolism (*e.g.*, proteins), and phosphate solubilization (Hungria et al., 2010). *A. brasilense*, in particular, can rapidly colonize maize roots in the early stages of crop development, increasing the activity of nitrogenases, which are important for the breakdown of triple bonds in the reduction of atmospheric N_2 to ammonia (NH_3). Ammonia can then be converted to ammonium (NH_4^+) and, subsequently, assimilated by plant cells (Sangoi et al., 2015; Brusamarello-Santos et al., 2017).

During root colonization, *A. brasilense* promotes structural changes to hosts, changes that are essential for plant development, such as an increase in root volume and length and adventitious root formation (Bashan et al., 2004; Calzavara et al., 2018). Such structural modifications allow greater contact between roots and soil, improving water and nutrient absorption and increasing the tolerance of plants to unfavorable conditions (Mehnaz, 2015). These modifications occur in response to auxin production and release promoted by *A. brasilense* colonization; auxin is responsible for cell division, extension, and differentiation (Puente et al., 2018).

In this study, higher N accumulation was observed in the grains and leaves of maize inoculated with *A. brasilense*, indicating greater N absorption capacity in treated plants. With the increased N absorption, there is also an increase in protein concentrations in plant tissues (Galeano et al., 2019). Proteins participate in various synthetic pathways, including those associated with resistance to adverse environmental conditions, resistance to pathogens, and improvement of photosynthetic capacity (Sharma & Dubey, 2019), ultimately resulting in yield gains.

In all studied localities, maize yield increased with treatment, although there were differences between localities. This finding is attributed to differences in edaphoclimatic conditions, which interfere with crop adaptation to the field (Silva et al., 2021). Nevertheless, *A. brasilense* was able to improve the productive, vegetative, and nutritional characteristics of maize grown under different conditions.

Grammy Crop® was effective in increasing maize yield compared with a commercial product and N fertilization. For grasses, *Azospirillum* spp. inoculation can replace N fertilization, even in soils with low organic carbon content and of different textures (Lana et al., 2012; Quatrin et al., 2019). These microorganisms help improve the sustainability of the cropping system by promoting the recovery of degraded soils and inducing plant tolerance to biotic and abiotic stresses (Hungria et al., 2016; Pankievicz et al., 2021).

Given that N fertilizers are responsible for a large share of greenhouse gas emissions in agriculture, the use of *A. brasilense* may help reduce this negative effect. Research has shown that it is possible to avoid the emission of 4.95 million Mg of CO_2 equivalents per year by replacing only 25% of mineral N with *A. brasilense* applied via seed treatment (Hungria et al., 2022).

Overall, the findings demonstrated that inoculation of maize seeds with *A. brasilense* can be considered an economically viable and sustainable strategy for supplying N to maize crops, contributing to plant development and yield (Zeffa et al., 2019).

5. Conclusion

All methods of application of Grammy Crop® (*A. brasilense*) were efficient in increasing maize vegetative growth and yield, as well as grain and leaf N contents, in all studied localities compared with the untreated control. Grammy Crop® afforded similar results to N fertilizer and a commercial *A. brasilense*-based inoculant.

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