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Azospirillum brasilense associated with nitrogen fertilization promotes improvement in macronutrient contents of maize plants

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Abstract

The lack of studies on the benefits of growth-promoting bacteria associated with sources and doses of nitrogen fertilizers in maize in tropical regions has raised many doubts on the use of nitrogen management, as well as recommendation for the adequate dose for maize production. Therefore, the objective of this study was to evaluate the effect of the doses and sources of nitrogen associated with the absence and presence of seed inoculated with *Azospirillum brasilense* in the contents of N, P, K, Ca, Mg and S in the roots, culm and leaf of maize plants. It was used a completely randomized design, in a 4 x 2 x 2 factorial scheme, consisting of four nitrogen doses (0; 60; 120; and 180 kg ha^{-1} of N), two sources of N (common urea and urea treated with urease inhibitor) and absence and presence of inoculation with *Azospirillum brasilense*, with four replications. The results identified that the N doses positively influenced the concentration of N, P, and S in the culm of maize plants. The use of urea with urease inhibitor was not efficient in optimizing nitrogen fertilization, providing similar accumulations of macronutrients to common urea. The inoculation with Azospirillum brasilense promoted a concentration of 10.4% of N in the stem when compared to urea without inoculation. Seed treatment with *Azospirillum brasilense* at a concentration of 1 g / kg of seed at a dose of 180 kg ha-1 of N provided a higher content of macronutrients in corn, regardless of the sources of common urea or urea treated with a urease inhibitor.

Keywords: diazotrophic bacteria, nutritional content, soil microorganism, urease inhibitor, *Zea mays* L.
Abbreviations: ATP_adenosine triphosphate; NBPT_urease inhibitor enzyme; ROS_reactive oxygen species.

Introduction

Maize (*Zea mays* L.) is a cereal of great economic importance in the global agricultural scenario. It is used mainly in human and animal nutrition. In the 2018/2019 crop, the Brazilian production covered an estimated cropped area of 17,254 million hectares, placing the country as the world's third-largest producer, preceded by the United States and China, in addition to being the second-largest grain exporter (Conab, 2019).

Nitrogen is the most limiting and costly nutrient in the maize production process. Along with rice and wheat, it uses around 50% of all nitrogen fertilizers (Galindo et al., 2020), in which supplementary N fertilization is essential to fulfill the requirements of non-leguminous crops over their production cycle (Teixeira Filho et al., 2014). Thus, quantification of the appropriate nitrogen doses and the improvement of nitrogen fertilizer management become an important agronomic decision that producers must take, especially because costs with fertilization are usually high (Galindo et al., 2019), to maximize production with the focus on sustainable development.

In Brazil, to achieve high yields of maize grains, high doses of nitrogen are applied as soils are highly weathered due to tropical climate and, consequently, large losses occur due to volatilization and ammonia leaching (GALINDO et al. 2020).

An alternative to reduce the losses caused by volatilization is the addition of urease inhibitors such as N- (n-butyl) thiophosphoric triamide (NBPT) to urea to reduce NH_3 volatilization (Manunza et al., 1999), which will increase the use efficiency of the applied nitrogen.

Another alternative to reduce costs of nitrogen fertilization is the treatment of seeds with inoculants containing growth-promoting bacteria (Hungria, 2011). Besides being economically viable and environmentally friendly, it represents a more sustainable pursuit of agriculture and increased efficiency in the use of nutrients in tropical farming (Galindo et al., 2020). Moreover, soil microorganisms such as *Azospirillum brasilense*, play a fundamental role in the sustainable development of ecosystems, allowing atmospheric nitrogen to be incorporated into the soil and the production and exudation of plant growth hormone such as auxins, gibberellins and cytokines in the root system (Alovisi et al., 2018).

Therefore, the hypothesis of this study is that inoculation with *Azospirillum brasilense* combined with nitrogen sources and doses could increase macronutrient assimilation in maize plants. Thus, the objective of this study was to evaluate the effect of nitrogen doses and sources associated with the absence and presence of seeds inoculated with *Azospirillum brasilense* on the concentration of N, P, K, Ca, Mg and S in the root, stem, and leaves of maize plants.

Results

Concentration of macronutrient in the corn leaf

The N doses showed an effect on the concentration of P, K, Mg, and S in the leaf tissue. For the P content (Table 2), in which the unfolding of the F × I interaction showed that the treatments urea in the presence of *Azospirillum brasilense* and urease inhibitor in the absence and presence of the bacteria were statistically equal, differing from urea treatment in the absence of inoculation, with values of 3.26, 3.40, 3.32 and 2.95 g kg^{-1}, respectively (Table 5). The interaction sources x doses showed a better adjustment of the quadratic equation, with maximum technical efficiency of 2.88 and 2.98 g kg^{-1} in the estimated doses of 122.5 and 149 kg ha^{-1} for conventional urea and urease inhibitor - NBPT, respectively (Table 7).

An effect was observed in the concentration of K in the leaf tissue, adjusted to the decreasing linear model for fertilization with urea without and with the inoculation, and urea with urease inhibitor -NBPT without *Azospirillum brasilense*. In relation to urea with urease inhibitor without the bacteria, the quadratic response was adjusted with maximum technical efficiency of 6.8 g kg^{-1} of K at the estimated dose of 137.1 kg ha^{-1} of N (Table 3).

The application of N influenced the Mg concentration in the leaf tissue, where the results did not fit any mathematical model, with an average of 1.64 and 1.87 g kg^{-1} for corn with urea and 1.88 and 1.64 g kg^{-1} and for the urease inhibitor at dose 0 and 180 kg ha^{-1} of N, respectively (Table 7).

For the concentration of S in maize plants, the statistical analysis showed an effect on the leaf, in which the treatment urea in the absence and presence of *Azospirillum brasilense* adjusted the quadratic function with maximum technical efficiency of 0.70 and 0.69 g kg^{-1} at the estimated doses of 105 and 145 kg ha^{-1} of N, respectively. The application of the urea source treated with the urease enzyme inhibitor without and with the bacteria provided a better fit to the decreasing linear model (Figure 3)

Concentration of macronutrient in the stalk of maize

The analysis of variance showed interaction for the N concentration in the stem of the maize plant (Table 2), in which the unfolding of the interaction revealed that the treatments urea with the presence of *Azospirillum brasilense* and urease inhibitor in the absence and presence of the bacteria were statistically equal, differing only from the treatment urea without inoculation, with the values of 3.18; 3.40; 3.01 and 2.88 g kg^{-1}, respectively (Table 5). For the isolated factor, the N doses showed the best fit for the increasing linear equation, in which the increase in nitrogen fertilization promoted the accumulation of the nutrient in the maize stalk, ranging from 2.69 to 3.43 g kg^{-1} at the doses 0 to 180 kg ha^{-1} of N, respectively (Table 4).

The doses of N significantly influenced the concentration of phosphorus in the maize stalk, showing the effect of the dose factor, with a quadratic response, in which the maximum technical efficiency of 2.71 g kg^{-1} P was obtained in the dose of 141.66 kg ha^{-1} of N (Table 4).

Nitrogen fertilization influenced the concentration of K in the culm of the maize plant, with an isolated effect of the doses presenting a decreasing linear response (Table 4).

In relation to calcium, the analysis of variance showed an interaction for the Ca concentration in the culm of the maize plant (Table 2). Also, regarding the culm, an effect was observed with an increasing linear response to simple urea, while the urease inhibitor did not present any adjustment to the mathematical models, with a mean value of 2.57 and 2.80 g kg^{-1} at dose 0 and 180 kg ha^{-1} of N, respectively (Table 7).

The N rates influenced the S content in the culm of the maize plant, in the case of the interaction, the unfolding means showed that the treatments urea with and without *Azospirillum brasilense* and urease inhibitor in the absence of the bacteria were statistically equal, differing only from the treatment urease inhibitor without inoculation (Table 5), and for the factor isolated doses, there was an increasing linear response.

Concentration of macronutrient in the root of corn

Regarding the concentration of N in the root, no effect was found for the factors isolated doses and sources, showing adjustment for the decreasing linear equation, regardless of the N sources applied (Table 7).

The effect of the interaction was observed for the content of P, K, Ca, and Mg in the root. Phosphorus showed the best fit to the quadratic equation, with maximum technical efficiency of 1.3 g kg^{-1} of P obtained at the dose of 77.5 kg ha^{-1} of N, fertilized with urea without inoculation. The application of urea with *Azospirillum brasilense* did not result in any adjustment to any mathematical model, with a mean of 1.3 and 1.1 g kg^{-1} at dose 0 and 180 kg ha^{-1} of N respectively, while urea treated with urease inhibitor - NBPT in the absence and presence of bacteria resulted in a better fit to the decreasing linear equation (Table 3).

The increment in N doses influenced the K content in the root tissue, the K in the root, a decreasing linear response was observed for the treatment with urea without *Azospirillum brasilense;* however, the application of urea with seed treatment and urease inhibitor in the absence and presence of *Azospirillum* provided a better adjustment to the quadratic equation, with maximum efficiency technique of 3.69, 1.40 and 2.80 g kg^{-1} of K obtained at the doses of 0; 121.3 and 34.2 kg ha^{-1} of N, respectively (Table 3).

Regarding the absorption of calcium by the root system, the increment in the N doses influenced the Ca content however, Urea in the absence of *Azospirillum brasilense* did not provide any adjustment to any mathematical model, with an average of 1.4 and 1.3 g kg-1 at the dose of 0 and 180 kg ha^{-1} of N, respectively, while urea with the bacteria promoted a better adjustment in the quadratic equation with maximum technical efficiency of 1.6 g kg^{-1} at the estimated dose of 71.4 kg ha^{-1} of N. For urea treated with NBPT in the absence and presence of inoculation of the bacteria, it presented an adjustment to the decreasing linear equation (Table 3).

Table 1. Soil physical and chemical analysis before sowing of maize plants.

Depth	N-total	pH	OM	C	P	K	Ca	Mg	Al	H+Al	SB
(cm)	kg ha^{-1}	$CaCl_2$	-----g dm^{-3}-----		mg dm^{-3}	-----mmol$_c$ dm^{-3}------			%	-----mmol$_c$ dm^{-3}-------	
0-20	4960	5.4	36	21	127.2	1.5	48	11	0	29	124
	CEC	V	Cu	Fe	Mn	Zn	B	S	Silt	Clay	Total sand
	mmol$_c$ dm^{-3}	%	-----------------------mg dm^{-3}------------------------						-----------g kg^{-1} -----------		
	153	68	1.6	37	36.3	52	0.59	16	99	136	765

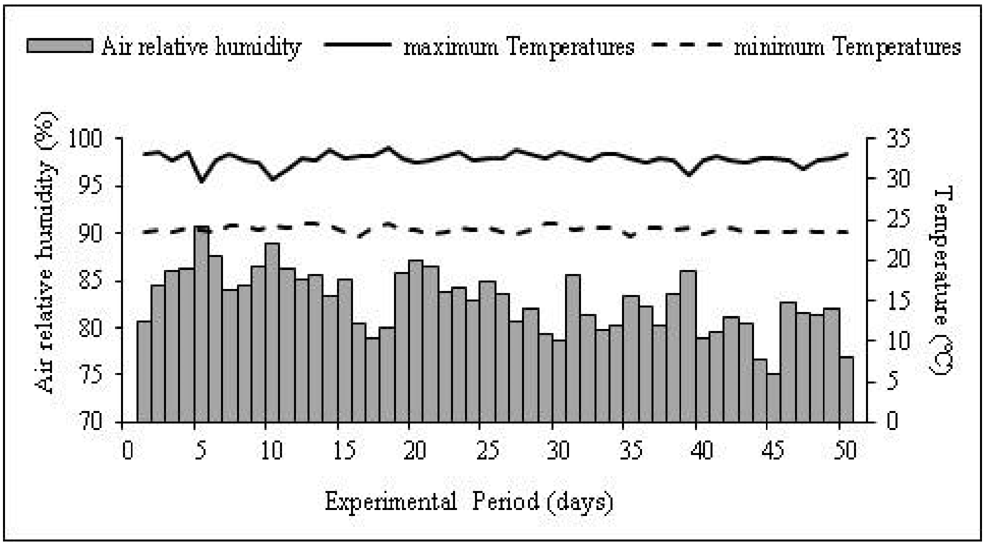

Figure 1. Air relative humidity and maximum and minimum temperature in the experimental area over the experimental period in 2019, Belém, State of Para, Brazil.

Table 2. Summary of analysis of variance, applied to the content of macronutrients (N, P, K, Ca, Mg and S) in the leaf, culm and root of maize hybrid, according to the nitrogen (D) doses, *Azospirillum brasilense* inoculation (I) and nitrogen sources (F).

Coefficient of variation	DF	Mean squares																	
		Plant organ																	
		Leaf						Culm						Root					
		Nutrient (g kg^{-1})																	
		N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
Sources (S)	1	0.02^{ns}	0.06*	5.34^{ns}	0.01^{ns}	0.02^{ns}	0.01^{ns}	0.01^{ns}	1.29^{ns}	0.84^{ns}	0.08^{ns}	0.04^{ns}	0.01^{ns}	0.01*	0.11*	0.01^{ns}	20.98*	0.03*	0.01^{ns}
Inoculation (I)	1	0.01^{ns}	0.02^{ns}	3.84^{ns}	0.01^{ns}	0.01^{ns}	0.04*	0.01^{ns}	0.06^{ns}	0.55^{ns}	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.01*	2.03^{ns}	0.01*	0.01^{ns}
Doses (D)	3	0.02^{ns}	0.13*	92.79*	0.01^{ns}	0.01^{ns}	0.03*	0.03*	7.88*	2.04*	0.13^{ns}	0.05^{ns}	0.09*	0.01*	0.07*	0.08*	2.85*	0.05*	0.54*
S x I	1	0.02^{ns}	0.04*	0.01^{ns}	0.01^{ns}	0.02^{ns}	0.01^{ns}	0.04*	0.27^{ns}	1.42^{ns}	0.02^{ns}	0.14^{ns}	0.21*	0.01^{ns}	0.02*	0.01^{ns}	13.51*	0.01^{ns}	0.04*
S x D	3	0.01^{ns}	0.03*	2.78^{ns}	0.01^{ns}	0.03*	0.01*	0.01^{ns}	1.07^{ns}	0.10^{ns}	0.33*	0.09^{ns}	0.01^{ns}	0.01^{ns}	0.04*	0.01*	2.54^{ns}	0.01*	0.01*
I x D	3	0.03^{ns}	0.01^{ns}	2.92^{ns}	0.01^{ns}	0.01^{ns}	0.01*	0.01^{ns}	0.34^{ns}	0.25^{ns}	0.05^{ns}	0.05^{ns}	0.01^{ns}	0.01^{ns}	0.02*	0.02*	1.55^{ns}	0.01*	0.01*
S x I x D	3	0.01^{ns}	0.01^{ns}	6.48*	0.01^{ns}	0.01^{ns}	0.01*	0.01^{ns}	0.10^{ns}	0.09^{ns}	0.15^{ns}	0.04^{ns}	0.03^{ns}	0.01^{ns}	0.01*	0.01*	4.12*	0.01*	0.01^{ns}
Blocks	3	0.03^{ns}	0.01^{ns}	9.81*	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.10^{ns}	0.37^{ns}	0.04^{ns}	0.01^{ns}	0.02^{ns}	0.01^{ns}	0.01^{ns}	0.01^{ns}	1.41^{ns}	0.01^{ns}	0.01^{ns}
Residue	45	0.02	0.01	2.21	0.01	0.01	0.01	0.01	0.76	0.39	0.07	0.04	0.03	0.01	0.01	0.01	0.01	0.01	0.01
Mean	-	9.8	3.2	10.3	3.2	1.7	0.7	3.1	3.1	6.6	2.6	2.7	0.3	6.2	1.3	2.3	1.5	0.8	0.7
CV (%)	-	5.9	8.9	17.6	1.1	21.4	20.4	10.9	23.9	25.9	17.7	18.2	16.4	2	11.2	4	7.1	23.1	15.1

ns: not-significant, *: significant at 5% probability by the F test, CV: coefficient of variation, DF: degree of freedom.

Table 3. Unfolding of the sources x inoculation x doses interaction, with regression equation and estimate of maximum technical efficiency, applied to the concentration of potassium (K), sulfur (S), calcium (Ca) in the leaf and potassium (K), phosphorus (P), magnesium (Mg) and calcium (Ca) in the roots of in maize hybrid without and with inoculation with *Azospirillum brasilense*.

Nutrient (g kg^{-1})	Plant organ	N source	*Azospirillum brasilense* inoculation	Dose (kg ha^{-1})				Equation	R^2	Ymet	Nmet
				0	60	120	180				
K	Leaf	Urea	Absence	12.6	10.6	8.1	7.5	$y=-0.0295x+12.4$	0.95	-	-
			Presence	14.2	10.8	8.6	7.4	$y=-0.0373x+13.657$	0.95	-	-
		Inhibitor	Absence	16.5	9.2	7.9	8.0	$y=0.0005x^2-0.1371x+16.276$	0.98	6.80	137.10
			Presence	13.7	10.9	10.8	8.4	$y=-0.0269x+13.403$	0.91	-	-
S		Urea	Absence	0.8	0.7	0.6	0.7	$y=0.00001x^2-0.0021x+0.8136$	0.94	0.70	105.00
			Presence	0.9	0.7	0.7	0.7	$y=0.00001x^2-0.0029x+0.9083$	0.80	0.69	145.00
		Inhibitor	Absence	0.8	0.7	0.7	0.7	$y=-0.0006x+0.8106$	0.59	-	-
			Presence	0.8	0.7	0.9	0.7	$y=0.8165$	-	-	-
K	Root	Urea	Absence	2.8	2.1	2.1	1.6	$y=-0.0061x+2.765$	0.86	-	-
			Presence	3.6	3.0	2.0	1.4	$y=-0.000006x^2-0.0117x+3.6961$	0.98	3.69	0.00
		Inhibitor	Absence	3.5	1.7	1.5	1.8	$y=0.0001x^2-0.0349x+3.5284$	0.97	1.40	121.30
			Presence	2.8	2.4	2.7	1.3	$y=-0.00007x^2+0.0048x+2.7818$	0.80	2.80	34.20
P		Urea	Absence	1.2	1.3	1.3	1.1	$y=-0.00002x^2+0.0031x+1.2465$	0.99	1.30	77.50
			Presence	1.3	1.4	1.2	1.3	$y=1.3446$	-	-	-
		Inhibitor	Absence	1.7	1.3	1.2	1.0	$y=-0.0036x+1.6847$	0.91	-	-
			Presence	1.4	1.4	1.1	1.1	$y=-0.0022x+1.4976$	0.86	-	-
Mg		Urea	Absence	0.7	0.8	0.8	0.7	$y=-0.000007x^2+0.0013x+0.7973$	0.99	0.85	92.85
			Presence	0.8	0.9	0.8	0.6	$y=-0.00002x^2+0.002x+0.8872$	0.98	0.93	50.00
		Inhibitor	Absence	0.9	0.9	0.8	0.7	$y=-0.0015x+1.0067$	0.98	-	-
			Presence	0.9	0.9	0.8	0.8	$y=-0.001x+0.9921$	0.75	-	-
Ca		Urea	Absence	1.4	1.5	1.4	1.3	$y=1.4615$	-	-	-
			Presence	1.6	1.5	1.3	1.2	$y=-0.000007x^2-0.001x+1.6285$	0.94	1.60	71.40
		Inhibitor	Absence	1.7	1.6	1.5	1.4	$y=-0.0016x+1.763$	0.95	-	-
			Presence	1.6	1.5	1.5	1.4	$y=-0.0009x+1.648$	0.88	-	-

R^2 - coefficient of determination; Ymet - estimated value of maximum technical efficiency; Nmet - nitrogen dose for maximum technical efficiency.

Table 4. Unfolding of the effect of nitrogen doses, with regression equation and estimation of maximum technical efficiency applied to the concentration of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) in the culm of maize plants subject to different nitrogen doses.

Nutrient (g kg^{-1})	Dose (kg ha^{-1})				Equation	R^2	Ymet	Nmet
	0	60	120	180				
N	2.69	3.02	3.30	3.43	y=0.0042x+2.7428	0.96	-	-
P	3.91	3.14	2.71	2.82	$Y=0.00006x^2–0.017x+3.9217$	0.99	2.71	141.66
K	8.51	6.29	6.68	5.09	y=-0.0164x+8.1286	0.80	-	-
S	0.32	0.31	0.36	0.36	y=0.0003x+0.3173	0.61	-	-

R^2 - coefficient of determination; Ymet – estimate value of maximum technical efficiency; Nmet –nitrogen dose by the estimate value of maximum technical efficiency.

Table 5. Summary of the analysis of the sources x inoculation interaction, according to the absence and presence of *Azospirillum brasilense* in maize seeds, at the concentration of nitrogen (N) in the culm, phosphorus (P) in the leaf, sulfur (S) in the culm, sulfur (S) in the root, in corn plants.

N source	*Azospirillum brasilense* inoculation	Plant organ		
		Leaf	Culm		Root
		Nutrient (g kg^{-1})			
		P	N	S	S
Urea	Absence	2.95±0.46 Bb	2.88±0.72 Ba	0.32±0.07 Ab	0.73±0.15 Bb
	Presence	3.26±0.42 Aa	3.18±0.63 Aa	0.37±0.06 Aa	0.76±0.13 Aa
Urease-inhibitor	Absence	3.40±0.65 Aa	3.40±0.75 Aa	0.36±0.07 Aa	0.77±0.16 Aa
	Presence	3.32±0.53 Aa	3.01±0.65 Aa	0.32±0.07 Ba	0.71±0.13 Bb

Columns with different capital letters between N-source treatments (urea and urease inhibitor - NBPT under the same inoculation treatment) and lowercase letters between inoculation treatments (absence and presence of *Azospirilum brasilense* under the same N source) indicate significant differences by the test of Tukey (P <0.05). Described values correspond to the mean of four repetitions and Standard Deviation.

Table *6*. Unfolding of the analysis of variance with regression equation and estimate of maximum technical efficiency, applied to the concentration of sulfur (S) in the root in maize hybrids, subject to the absence and presence of *Azospirillum brasilense* and to the doses of nitrogen.

Nutrient (g kg^{-1})	*Azospirillum brasilense* inoculation	Dose (kg ha^{-1})				Equation	R^2	Ymet	Nmet
		0	60	120	180				
S	Absence	0.95	0.79	0.67	0.56	y=-0.0021x+0.9412	0.99	-	-
	Presence	0.86	0.84	0.67	0.56	$y=-0.000006x^2–0.0006x+0.8706$	0.96	0.88	50.0

R^2 - coefficient of determination; Ymet – estimate value of maximum technical efficiency; Nmet – nitrogen dose through the maximum technical efficiency.

Table 7. Unfolding of sources x doses interaction, with regression equation and estimate of maximum technical efficiency applied to the concentration of nitrogen (N) in the roots, phosphorus (P) in the leaf, calcium (Ca) in the stem, magnesium (Mg) in the leaf, sulfur (S) in the root in corn hybrid seed submitted to different nitrogen sources.

Nutrient (g kg^{-1})	Plant organ	N sources	Dose (kg ha^{-1})				Equation	R^2	Ymet	Nmet
			0	60	120	180				
P	Leaf	Urea	3.46	3.11	2.8	3.02	$y=0.00004x^2–0.0098x+3.4891$	0.94	2.88	122.5
		Inhibitor	4.15	3.19	3.17	2.9	$y=0.00005x^2–0.0149x+4.0951$	0.91	2.98	149.0
Mg		Urea	1.64	1.64	1.69	1.87	y=1.7131	-	-	-
		Inhibitor	1.88	1.78	1.92	1.64	y=1.8091	-	-	-
Ca	Culm	Urea	2.41	2.28	2.98	2.88	y=0.0035x+2.3272	0.62	-	-
		Inhibitor	2.57	2.66	2.57	2.8	y=2.6541	-	-	-
N	Root	Urea	7.28	7.07	6.29	5.68	y=-0.0093x+7.4236	0.95	-	-
		Inhibitor	6.61	5.92	5.8	5.48	y=-0.0059x+6.4831	0.9	-	-
S		Urea	0.90	0.83	0.65	0.59	y=-0.0019x+0.913	0.96	-	-
		Inhibitor	0.90	0.81	0.7	0.54	$y=-0.000005x^2–0.0012x+0.9046$	0.99	0.97	120.0

R^2 - coefficient of determination; Ymet – estimate value of maximum technical efficiency; Nmet – nitrogen dose though maximum technical efficiency.

N doses promoted interaction in the concentration of Mg in the root tissue, with a quadratic response, with the maximum technical efficiency of 0.85 and 0.93 g kg^{-1} obtained at doses of 92.85 and 50.00 kg ha^{-1} of N for urea in the absence and presence of *Azospirillum brasilense* respectively, whereas in the source with urease inhibitor without and with the inoculation of the bacteria resulted in an adjustment to the decreasing linear model (Table 3).

The analysis of variance showed the effect for the concentration of S in the root (Table 2), in which the interaction unfolding showed that the treatments urea in the presence of *Azospirillum brasilense* and urease inhibitor in the absence of the bacteria were statistically equal, differing only from the treatments urea in the absence of inoculation and inhibitor of the enzyme urease in the presence of the bacterium, with the values of 0.76, 0.77, 0.73 and 0.71 g kg^{-1}, respectively (Table 5). For interaction inoculation x doses, there was a decreasing linear response to urea in the absence of *Azospirillum brasilense*, and a quadratic response with maximum technical efficiency of 0.88 g kg^{-1} at a dose of 50 kg ha^{-1} of N for the concentration of S, with the application of urea treated with a urease inhibitor in the presence of the bacteria (Table 6). On the other hand, the source x dose interaction showed a decreasing linear response in the case of urea and a quadratic response for the urease inhibitor, with a maximum value of 0.97 g kg^{-1} at a dose of 120 kg ha^{-1} of N (Table 7).

Discussion

Concentration of macronutrient in the corn leaf

The values related to levels of phosphorus and magnesium in the corn leaf are within the ideal range recommended for the crop (Cantarella et al., 1997), between 2.0 - 4.0 and 1.5 - 5.0 g kg^{-1} respectively (Table 5). Regardless of the sources and doses of N tested, while the average concentration of potassium and sulfur in the leaf was below the recommended ideal for corn, which ranges from 17 to 35 and 1.5 to 3 g kg^{-1} respectively (Table 3) (Cantarella et al., 1997). Phosphorus is one of the nutrients most demanded by corn plants. It accounts for the development and yield of the crop (Dhillon et al., 2017), thus the adequate availability of P promotes the initial development of the root system, improving plant growth, playing a fundamental role in plant nutrition and development (Lollato et al., 2019).

For Mg, the adequate content in plants is very important for the success of the corn harvest, as it is an essential nutrient for the conformational stabilization of macromolecules (Galindo et al., 2020).

The K content in the leaf decreased as N doses increased, Similar results were reported by Vasconcelo et al. (2016) when studying the content of K in the leaves of corn in association with doses of N and inoculation with *Azospirillum brasilense.* Regarding the S content in the leaf, it was observed that there was no effect of the increase of N in the accumulation of S in the corn leaf. Longhini et al. (2016) found that the doses of N did not influence the concentration of S in the leaves of corn, being below the critical levels.

Concentration of macronutrient in the stalk of maize

The accumulation of nitrogen in the culm of maize plants inoculated with the bacteria can be attributed to the process of biological N fixation and the secretory function of growth hormone as auxin, which acts by stimulating the growth of root hair, promoting nutrient absorption (Alovisio et al., 2018). For Coelho et al. (2018), nitrogen loss can be reduced with the application of urea treated with a urease enzyme inhibitor, due to the decrease in ammonium volatilization in the soil, therefore increasing the availability of N for plant growth.

The increment in N doses influenced the concentrations of P, S, K, and Ca in the culm of the maize plant. it was found that the increase in nitrogen fertilization promoted accumulation of P and S in the stalk of the maize plant. An inverse relationship was observed for K concentration. The results showed the importance of N in the nutrition and nutrient absorption in maize plants (Galindo et al. 2016). Thus, the greater availability of N for plants may have favored the development of the root system which, when exploring a larger volume of soil, absorbed greater amounts of nutrients (Vettorazzi et al., 2019).

Regarding the S accumulation in the culm of maize plants, Galindo et al. (2016) found in the plants grown from seeds inoculated with the bacteria, a higher S concentration in the aerial part than that in non-inoculated plants, caused by the greater CO_2 fixation, which increases the capacity to rise biological nitrogen fixation and positively influence the metabolism of C4 plants, which is closely related to the metabolism of N assimilation in the plant.

Regarding the K content in the stem, it was observed that the nutrient was negatively affected by nitrogen fertilization. Batista and Monteiro (2010), when evaluating the nutritional status of Marandu grass observed a reduction in the concentration of K in the sprouts of the species. However, Petean et al. (2019) reported that the potassium level in the leaves was positively affected by nitrogen fertilization, contradicting the results obtained in this study.

For the Ca concentration in the culm of maize plants, simple urea was more efficient in calcium absorption compared to the urease inhibitor - NBPT, mainly at the highest doses (Table 7), possibly because urea releases N into the soil more rapidly, while the urease inhibitor reduces the availability of N for a particular period (Galindo et al., 2020).

Concentration of macronutrient in the root of corn

The reduction in the concentration of N, P, K, Ca, Mg and S in the root system as a result of nitrogen fertilization, Galindo et al. (2016) stated that the effect may be associated with translocation of nutrients to the aerial part of the plant since there is a threshold on the nutritional demand of the plant, that is, the vegetables absorb only a part of the available nutrients and the rest can be lost via leaching and volatilization. Furthermore, the fact that there are several microorganisms in the soil with a high affinity for available mineral nutrients (Barraclough et al., 2010).

Materials and methods

Area characterization and soil analysis

The experiment was conducted in a greenhouse, located at the Institute of Agricultural Sciences of the Federal Rural University of the Amazon, Belém, Brazil within the following geographic coordinates: 48°26’18.0” west longitude and 1°27’17.3” south latitude. The climate in the area is of the Afi-type according to the Köppen classification. The sandy-loam soil used in the experiment was collected at the 0-20 cm depth, classified as a dystrophic Yellow Latosol (Embrapa, 2018). Afterward, composite samples were taken to the Brazilian Institute of Analysis for physical and chemical analysis of the soil, according to the methodology described by (Silva, 1999). It can be seen in Table 1 the need for potassium correction with the application of 60 kg ha^{-1} of KCl (Oliveira et al., 2018). Climatic data from the experimental area were collected during the conduction of the experiment (Figure 1).

Experimental design and experiment conduction

The seed used in the experiment was K9960 VIP3 corn hybrid, usually adopted in the southeastern region of the State of Pará. After collection, the soil was passed through a 2-mm sieve, then 768 kg of soil was homogenized in 256 kg of organic matter from mango pruning residues. The 25 x 32 cm pots were filled with 16 kg substrate at the proportion of 3:1, respectively.

It was used a completely randomized block design, in a 4x2 x2 factorial scheme, as follows: four doses of N (0; 60; 120 and 180 kg ha^{-1} N), two sources of N, common urea (with 45% de N) and urea treated with urease inhibitor enzyme - NBPT (with 45% N) and presence and absence of inoculation with *Azospirillum brasilense*, with four replications.

For the treatments with inoculation, the seeds were homogenized together with the inoculant (200 mL diluted in water equivalent to 10% of the weight of the seeds at the concentration 2×10^8 CFU mL^{-1}), inoculated one hour before sowing (Leite et al., 2019). Nitrogen doses were applied in a single dose in topdressing, performed 10 days after the emergence of the plants following the recommendation of (Jadoski et al., 2016).

The buckets received controlled daily irrigation to replace the evapotransporated water during the experimental period, and the soil water content was maintained at field capacity, using the gravimetric method (Catuchi et al., 2011). Weeds and pests were controlled manually and daily, through mechanical pulling and manual picking, respectively.

Plant evaluation

The evaluations were carried out at the time of full male flowering, that is, tasseling (Ritchie et al.,1993). At 50 days after germination, the macronutrient concentration in the corn hybrid was measured.

Nutritional evaluation

For the determination of macronutrients, the material was washed in deionized water and dried in an oven with forced air circulation at 65 ± 2 ºC, until reaching constant mass. Next, the samples were weighed on an analytical balance to obtain dry matter, and ground in a Wiley mill, equipped with a 20-mesh sieve. Next, the materials were stored and identified and sent to the Plant Science Laboratory /Plant Mineral Nutrition sector, located at the State University of the North Fluminense Darcy Ribeiro- Rio de Janeiro, and the nitrogen (N) content was determined through sulfuric digestion using the Nessler's method (Jackson, 1965). Also, phosphorus (P), potassium (K), sulfur (S), magnesium (Mg), and calcium (Ca) were submitted to digestion with concentrated nitric acid and hydrogen peroxide in an open digestion system and plasma-quantified (ICPE-9000) of the Shimadzu® brand (Peters, 2005).

Statistical analysis

The analysis of variance was performed and the significant unfolding were also carried out. To evaluate the effect of nitrogen doses, fertilizer sources, and inoculation on corn hybrid, the test of Tukey was used at 5% probability, using the Sisvar statistical software (Ferreira, 2019).

Conclusion

The increase in the doses of N positively influenced the concentration of N, P, S in the culm of maize plants. Urea treated with a urease inhibitor is not efficient in optimizing nitrogen fertilization, providing similar accumulations of macronutrients compared to simple urea, particularly for the levels of P in the leaf; N, Ca, and S in the stem and K and S in the root. Inoculation with *Azospirillum brasilense* promoted a higher concentration of N in the stem, and Ca and Mg in the root. Nitrogen fertilization reduced the macronutrient concentration in the root of maize plants, regardless of the sources of N and the seed inoculation with *Azospirillum brasilense*. The dose of 180 kg ha^{-1} of N associated with seed inoculation with *Azospirillum brasilense* provided a higher concentration of macronutrients in corn, regardless of the application of common urea or urea treated with a urease inhibitor.

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