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Inoculation efficiency of *Azospirillum brasilense* on economising nitrogen fertiliser in landrace popcorn[1]

Eficiência da inoculação com *Azospirillum brasilense* na economia de fertilizantes nitrogenados em milho pipoca crioulo

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ABSTRACT - Nitrogen is one of the nutrients most demandedby popcorn, and one that most increases production costs. Considering these factors, the aim of this study was to evaluate the effectiveness of inoculatingpopcorn seeds with *Azospirillum brasilense* together with four levels of nitrogen fertiliser, and its contribution to characteristics related to production. The experiment was carried out in the experimental area of the Centre for Agrarian Sciences and Biodiversity of the Federal University of Cariri, in Crato, in the State of Ceará. Eight treatments were evaluated with four replications, using an experimental design of randomised blocks in a 2x4 factorial scheme corresponding to the presence and absence of inoculation, and to four doses of nitrogen as top dressing. The following characteristics were evaluated: plant height, height of ear insertion, number of plants, number of lodged plants, number of broken plants, number of poorly husked ears, ear diameter, ear length, number of ears, number of diseased ears, number of infested ears, grain productivity, ear weight, mean 100-grain weight, and expansion capacity. There were significant differences for most of the characteristics under evaluation, with an increase of 17.18% in the number of ears and 10.78% in grain productivity, reducing the top dressing to 50 kg ha^{-1} in the presence of *A. brasiliensi*. These results demonstrate the potential for economisingnitrogen fertiliser in popcorn production under the soil and climate conditions of the Cariri region of the State of Ceará.

Key words: Diazotrophic bacteria. Biological nitrogen fixation. Nitrogen fertiliser.

RESUMO - O nitrogênio é um dos nutrientes mais exigidos pelo milho pipoca e também o que mais onera o custo de produção. Considerando-se esses fatores objetivou-se comesse trabalho avaliar a eficácia da inoculação com *Azospirillum brasilense* em sementes de milho pipoca, associada a quatro níveis de adubação nitrogenada e a sua contribuição para as características ligadas à produção. O experimento foi conduzido na área experimental do Centro de Ciências Agrárias e da Biodiversidade da Universidade Federal do Cariri- Crato, CE. Foram avaliados oito tratamentos com quatro repetições, utilizando-se o delineamento experimental em blocos ao acaso, em esquema fatorial 2x4 correspondente àpresença e ausência de inoculação, e a quatro doses de nitrogênio em cobertura. Foram avaliadas as características: altura da planta, altura da inserção da espiga, número de plantas, número de plantas acamadas, número de plantas quebradas, número de espiga mal empalhada, diâmetro de espiga, comprimento de espigas, número de espiga, número de espiga doente, número de espigas com praga, produtividade de grão, peso de espiga,peso médio de 100 grãos e capacidade de expansão.Houve diferenças significativas para a maioria das características avaliadas com um incremento de 17,18% para número de espiga e 10,78% para produtividade de grão reduzindo-se a adubação de cobertura a 50 kg ha^{-1} na presença de *A. brasiliensi*. Estes resultados demonstram o potencial na economia de adubos nitrogenados na produção de milho pipoca nas condições edafoclimáticas do Cariri Cearense.

Palavras-chaves: Bactéria diazotrófica. Fixação biológica do nitrogênio. Adubaçãonitrogenada.

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INTRODUCTION

Popcorn belongs to the species *Zea mays* L. var. everta. It differs from ordinary maize due to its small hard grains, which burst when heated to temperatures of around 180 ºC (SAWAZAKI, 2001). On the world scene, Brazil stands out as a major producer of popcorn with an annual production estimated at 80 thousand tons per year. However, low grain productivity in the production chain is the main obstacle to the crop being adopted in the country (MIRANDA *et al*., 2011). To this effect, it is believed that the development of cultivars adapted to individual growing regions is the most suitable way of solving this problem in the long term. In the short term, techniques that promote increases in productivity should be developed, and their applicability tested for the different genotypes. Among the sources of fertiliser, nitrogen (N) is one of the most required nutrients of the maize crop, and one that most increases production costs (OLIVEIRA *et al*., 2009; PEREIRA *et al*., 2015). Several studies have recently emphasised associating bacteria and/or fungi with plants in order to improve the relationship between soil and plant, and consequently the rate of nutrient absorption (QUADROS *et al*., 2014; REPKE *et al*., 2013; SANTOS *et al*., 2013). In this context, Biological Nitrogen Fixation (BNF), promoted by nitrifying bacteria among others, is seen as an important alternative for the supply and absorption rate of the nitrogen necessary to the crop, contributing to the sustainability of agricultural production systems (BERGAMASCHI *et al*., 2007; PEREIRA *et al*., 2015). According to Bashan and Bashan (2010), bacteria of the genus *Azospirillum* can be inoculated into plants of agronomic interest in order to increase the rate of N absorption. In addition, the use of microorganisms stimulates the development of multiple mechanisms that contribute to an increase in productivity, such as the synthesis of phytohormones, an increase in the root system, the mitigation of various stresses, and the biological control of pathogenic microbiota. However, according to Bartchechen *et al*. (2010), the main limitation on the use of this technology is the inconsistency of research results, which leads to variations in production according to genotype, soil and climate conditions, and study methodology.

Considering nitrogen fertiliser as one of the main factors to increase the costs of maize production, the aim of this study was to evaluate the effectiveness of inoculation with *A. brasilense* in popcorn seeds, together with four levels of nitrogen fertiliser, on characteristics related to production in the southern region of the state of Ceará.

MATERIAL AND METHODS

Location and characteristics of the experimental area

The experiment was carried out in the experimental area of the Centre for Agricultural Sciences and Biodiversity of the Federal University of Cariri (CCAB/UFCA), in the town of Crato, located at 7º14’03” S and 39º24’34”W, at an average altitude of 426.9 m, in the extreme south of the State of Ceará. The average annual rainfall is 1,090.9 mm, with rains concentrated from January to May and average temperatures varying between 24 and 26 ºC throughout the year (IPECE, 2012).

The soil in in the experimental area is classified as a dystrophic Red-Yellow Latosol. Granulometric analysis of the 0-0.20 m layer revealed an average content of 90 g kg^{-1} clay, 20 g kg^{-1} silt and 890 g kg^{-1} sand, the textural class being defined as loamy sand according to the Brazilian System of Soil Classification.

Inoculation

Initially, some of the maize seeds were inoculated with liquid commercial AzoTotal ®, based on *A. brasilense* (Strains AbV5 and AbV6), at a minimum concentration of 2.0 x 10^8 colony forming units per mL. The mixture was carefully prepared to ensure uniform distribution of the inoculant over the seeds. The applied dose was as recommended by the manufacturer, equivalent to 100 mL for 60,000 seeds. After inoculation, the seeds were protected from the sun for about 2 hours prior to sowing.

Plant material, planting, and cropping treatments

The landrace popcorn genotype ‘Iva’was used in the study, obtained from the germplasm of CCAB/UFCA. The design was of randomised blocks in a 2 x 4 factorial scheme with four replications. The first factor corresponded to the presence or absence of inoculation, and the second to the four doses of nitrogen top dressing (0, 50, 100 and 150 kg N ha^{-1}), resulting in eight treatments. The experimental plot consisted of rows, 5.00 m in length and spaced 0.90 m apart, with a planting depth of 0.05 m. Three seeds were sown per hole, spaced every 0.20 m. Thinning was carried out 15 days after planting, leaving only one plant per hole for a total of 25 plants per row.

Base fertilisation was carried out manually when sowing, applying 350 kg ha^{-1} 10:10:10 NPK in the planting row. Nitrogen fertiliser was applied at the V_6 stage, with applications varying according to the doses under study (0, 50, 100 and 150 kg N ha^{-1}), using 45% N urea as the source. Cover fertiliser was broadcast approximately 20 cm from the root collar. The ears were harvested around 120 days after sowing.

Evaluated characteristics

Plant height (PH) - measuring the distance in metres from the root collar to the insertion of the flag leaf; height of ear insertion (EH) - a measure of the length in metres from the base of the plant to the insertion of the first ear; number of plants (NP) - total number of plants in the plot when harvesting; number of lodged plants (NL) - considered lodged when the angle between the base of the stem and the ground was less than 45°; number of broken plants (NB) - considered broken, stems that showed significant rupture in the supporting tissue below the point of upper ear insertion; number of poorly husked ears (PE) - the number of poorly husked ears per plot; ear diameter (ED) - obtained with the aid of a digital calliper, in millimetres; ear length (EL) - measuring the distance in millimetres between the first and last grain on the longest row; number of ears (NE) - the number of ears harvested in each plot; number of infested ears (NI) - a count of the number of ears attacked by pests per plot; number of diseased ears (ND) - obtained by counting the diseased ears per plot; grain productivity (GP) - obtained from the weight of the grain without the cob, in kg ha^{-1}; ear weight (EW) - obtained by weighing the dehusked ears, in kg ha^{-1}; mean 100-grain weight (W100) – separating two replications of 100 grains per plot and weighing on a digital balance to obtain the weight of 100 grains; grain expansion capacity (EC) - the volume of the expanded popcorn, determined with the aid of a microwave oven, in which 30g of seeds in kraft paper bags were placed for two minutes and fifteen seconds, in four replications per experimental unit.

Statistical analysis

Eight treatments with four replications were evaluated using an experimental design of randomised blocks in a 2 x 4 factorial scheme, corresponding to two types of inoculation with *Azospirillum brasilense* (with and without inoculant), and four doses of nitrogen as top dressing. The GENES software (CRUZ, 2013) was used for a statistical analysis of the results. Analyses of variance were carried out in a factorial scheme, with the mean values compared using Tukey's test at 5% probability for the factor inoculation, and polynomial regression for the levels of fertiliser.

RESULTS AND DISCUSSION

Table 1, a summary of the analysis of variance, shows the mean-square significance, as well as the mean values and the coefficients of experimental variation for the fifteen characteristics under evaluation. In general, when evaluating the behaviour of the variation in characteristics, it can be seen that for the majority, the coefficient of variation (CVe%) was low, especially for characteristics related to production (GP, EW, W100 and NE), reflecting the experimental accuracy.

There were significant differences in the inoculant x fertiliser interaction for most of the characteristics under evaluation, except for number of plants, number of lodged plants, number of broken plants, poorly husked ears and number of infested ears. For these characteristics, the absence of a response to the stimulus of *A. brasiliense* or to the fertiliser was somewhat expected, since these characteristics are determined more by the crop environment than by the physiological mechanism of the plant itself (CRUZ *et al.*, 2006; MAGALHÃES; SOUZA, 2011).

Furthermore, when such characteristics as the number of diseased ears are observed, it can be seen that there was a significant difference between treatments,relative to inoculation as well as to expansion capacity. Bashan and Bashan (2010) point out the effects of *A. brasilense* as potentially reducing damage in plants subjected to such interactions, however it is believed that this effect occurs by an increase in the physiological functions of the plant due to the benefits caused by the bacterium. To date there is no understanding of how this relationship is established, nor whether the effect of disease is actually controlled or reduced due to inoculation with *Azospirillum*. Conversely, the number of diseased ears and expansion capacity did not show any variation for fertiliser dose.

The number of ears in the plot, judging from the significances of fertiliser dose and inoculant concentration, showed a notable increase; however, the interaction between the effects of the inoculant and fertiliser was somehow not significant. This fact cannot be completely explained; it is believed that both the fertiliser and inoculation with *A. brasilense* contribute to an increase in the number of ears per plant. However, judging by the mean values for number of ears, with and without inoculation, and by the increase in this characteristic shown in Table 2, it can be seen that inoculation with *Azospirillum*resulted in significant gains in the number of ears, including an increase of 23.07% in the number of ears even with a fertilizer concentration of zero.

For the attributes that showed a significant effect in the IF interaction, it can be seen that a part of the demand for N in the maize was met by the association with *A. brasilense*, resulting in benefits to the crop, such as higher and more vigorous plants, an increase in ear diameter and length, as well as an increase in the weight of the grain and ears, with a consequent increase in productivity.

Table 1 - Mean squares, mean values and coefficients of experimental variation for the evaluated characteristics in popcorn as a function of inoculation with *A. brasilense* (strains AbV5 and AbV6) associated with nitrogen fertiliser

SV	DF	Mean Square							
		PH	EH	NP	NL	NB	PE	ED	EL
Block	3	0.00153	0.0103	63.15	0.7395	0.3125	27.291	0.0086	0.246
Inoculant (I)	1	0.13520**	0.00911*	175.78ns	0.031ns	0.0ns	0.0ns	0.2628**	15.526**
Fertiliser (A)	3	0.37609**	0.13842**	54.28ns	0.281ns	0.541ns	0.041ns	0.5088**	28.190**
IF	3	0.04011**	0.01067**	44.36ns	0.197ns	0.25ns	0.25ns	0.0768**	4.504**
Residual	21	0.00228	0.00057	47.912	0.6503	0.6607	0.4464	0.00659	0.500
Gen. Mean		1.67	0.80	45.21	1.59	1.56	1.43	3.24	13.17
CVe(%)		2.94	3.19	15.31	50.60	52.02	46.48	2.24	5.59

SV	DF	Mean Square						
		NE	ND	NI	GP	EW	W100	EC
Block	3	70.416	0.5312	0.3229	2856.25	2472.92	0.9559	0.2176
Inoculant (I)	1	820.12**	34.031**	0.281ns	201612.50**	487578.13**	4.9455**	1.753*
Fertiliser (A)	3	420.37**	0.6979ns	1.364ns	623014.58**	422422.92**	2.6462**	0.592ns
IF	3	7.87ns	5.1979**	1.614ns	9277.08*	20071.88**	2.0088**	0.321ns
Residual	21	7.2798	0.3884	0.6205	2480.06	1558.036	0.28501	0.3417
Gen. Mean		52.68	3.21	3.46	1460.62	1648.75	16.15	26.90
CVe (%)		5.12	18.75	22.82	3.41	2.20	3.44	2.17

*Significant at a level of 5% probability; ** Significant at a level of 1% probability; ns = Not significant; IF = Inoculant/Fertiliser Interaction; CVe = coefficient of experimental variation; PH = Plant height; EH = Height of ear insertion; NP = Number of plants; NL = Number of lodged plants; NB = Number of broken plants; PE = Number of poorly husked ears; ED = Ear diameter; EL = Ear length; NE = Number of ears; ND = Number of diseased ears; NI = Number of infested ears; GP = Grain productivity; EW = Ear weight; W100 = 100-grain weight; EC = Expansion capacity

The characteristics of plant height, height of ear insertion, ear diameter, ear length, number of diseased ears, grain productivity, ear weight and 100-grain weight showed significant interaction for the effects of the inoculant x dose of fertiliser interaction,with linear, quadratic and cubic behaviour, as per the equations described in Table 3. These characteristics displayed wide variation as a function of the combinations of fertiliser and inoculant; however, for a better interpretation of the data, it would be appropriate to evaluate the data based on the mean values of the characteristics and the increase in these values caused by the fertiliser and/or inoculant.

For the PH characteristic, inoculation of the seeds with *A. brasiliense*with no nitrogen fertiliser gave an increase of 22.4%, and when associating the bacterium with the nitrogen, the fertilisation at 50 kg N ha^{-1}was noteworthy, resulting in plants of up to 1.73 m (Table 2), an increase of 13% in relation to the treatment with no inoculant. In the other treatments, the doses of N associated with the bacterium did not show any significant increase in plant height. The EH characteristic is also notable in the presence of the inoculant. These results agree with those seen in other studies, such as in Hungary *et al*. (2010), Kappes *et al*. (2013), Santos *et al*. (2013) and Wagatsuma *et al*. (2012).

Under field conditions, the speed of initial plant growth favours the crop by reducing the growth of weeds due to the effect of shading. However, it is worth emphasising that excessive plant height or ear insertion height may predispose the plant to lodging or breaking, resulting in a low final crop yield (SANTOS *et al*., 2013).

According to some authors, phytohormones excreted by *Azospirillum*, particularly indole-acetic acid (IAA), may play a fundamental role in promoting plant growth, especially during the early stages of development and the rooting process (BASHAN; HOLGUIN, 1997; PEDRINHO, 2009).

According to Pedrinho (2009), interaction between the plant genotype, the endophytic growth-promoting community and other factors, may affect plant development. However, although there are some studies that demonstrate the mechanism for endophytic microorganisms promoting plant growth, there is a lack of further studiesneeded for a better understanding of the processes involved.

Table 2 - Mean values and increases in inoculation with *A. brasilense* diazotrophic bacteria (strains AbV5 and AbV6) for fertiliser dose, in plants of landrace popcorn associated with nitrogen fertiliser

Dose	PH		I (%)	EH		I%	NP		I%
	N	W		N	W		N	W	
0	1.25 b	1.53 a	22.4	0.567 b	0.695 a	22.57	40.50	45.25	-
50	1.52 b	1.73 a	13.81	0.770 b	0.817 a	6.10	45.75	48.00	-
100	1.75 a	1.75 a	0	0.827 a	0.832 a	0.60	37.50	48.75	-
150	1.90 a	1.91 a	0.52	0.972 a	0.927 b	-4.62	47.75	48.25	-
Mean							42.87 a	47.56 a	
Dose	**NL**		**I%**	**NB**		**I%**	**PE**		**I%**
	N	W		N	W		N	W	
0	1.50	2.00	-	1.25	1.25	-	1.50	1.25	-
50	1.50	1.50	-	1.75	1.50	-	1.25	1.75	-
100	1.50	1.25	-	1.25	1.75	-	1.50	1.25	-
150	1.75	1.75	-	2.00	1.75	-	1.50	1.50	-
Mean	1.56	1.62	-	1.56	1.56		1.43	1.43	
Dose	**ED**		**I (%)**	**EL**		**I (%)**	**NE**		**I (%)**
	N	W		N	W		N	W	
0	2.75 b	3.15 a	14.54	9.13 b	12.55 a	37.45	39.00	48.00	23.07
50	3.02 b	3.28 a	8.6	12.18 a	13.18 a	8.21	48.00	56.25	17.18
100	3.26 b	3.40 a	4.29	13.25 b	14.59 a	10.11	48.75	59.25	21.53
150	3.58 a	3.51 a	-1.9	15.34 a	15.16 a	-1.17	54.75	67.50	23.28
Mean	-	-		-	-		47.625b	57.75a	
Dose	**ND**		**I (%)**	**NI**		**I (%)**	**GP**		**I (%)**
	N	W		N	W		N	W	
0	4.25 a	1.75 b	-	2.50	3.25	-	946.25 b	1187.5 a	25.49
50	4.50 a	2.00 b	-	4.25	3.25	-	1413.75 b	1566.25 a	10.78
100	3.50 a	3.75 a	-	3.50	3.50	-	1530.00 b	1605.00 a	4.90
150	4.75 a	1.25 b	-	3.25	4.25	-	1635.00 b	1801.25 a	10.16
Mean	-	-		3.37 a	3.56 a		-	-	
Dose	**EW**		**I (%)**	**W100**		**I (%)**	**EC**		**I (%)**
	N	W		N	W		N	W	
0	1097.50 b	1462.50 a	33.25	14.47 b	16.52 a	14.16	27.58	26.70	-
50	1565.00 b	1742.50 a	11.34	15.37 b	16.50 a	7.35	26.49	26.58	-
100	1630.00 b	1781.25 a	9.27	16.36 a	16.27 a	-0.55	27.33	26.83	-
150	1808.75 b	2102.50 a	16.24	16.82 a	16.88 a	-0.11	27.16	26.58	-
Mean	-	-		-	-		27.14 a	26.67 b	

Mean values followed by the same letter for each characteristic in the factor inoculation do not differ by Tukey's test at 5% probability. D = Nitrogen Dose (kg ha^{-1}). N = With no bacterium,*A. brasilense*; W = With the bacterium *A. brasilense*; I = Increase. PH = Plant height; EH = Height of ear insertion; NP = Number of plants; NL = Number of lodged plants; NB = Number of broken plants; PE = Number of poorly husked ears; ED = Ear diameter; EL = Ear length; NE = Number of ears; ND = Number of diseased ears; NI = Number of infested ears; GP = Grain productivity; EW = Ear weight; W100 = 100-grain weight; EC = Expansion capacity

For the attribute number of ears, the highest mean values were obtained in treatments that included inoculation with *A. brasiliense*. The increases varied between 17.18 and 23.28% (Table 2) in the presence of the inoculant associated with the different doses of N. It can be seen that the treatment with the inoculant associated with 150 kg of N fertiliser ha^{-1} was more pronounced, with an average of 67.50 ears. On the other hand, the treatment with the same amount of nitrogen fertiliser, but with no inoculation, displayed a lower average, 54.75 ears. Although the results of the ANOVA (Table 1) did not show significance for association of the bacteria withthe nitrogen fertiliser, a significant increase in the number of ears can be seen when such an association occurs, as per Table 2.

In addition to productivity and grain yield, other characteristics determine the productive potential of maize, such as ear diameter and ear length, which correlate directly with grain filling and the number of grain rows per ear. Under the conditions of the experiment, the attributes ED and EL were notable in the presence of the inoculant and in association with the nitrogen fertiliser.

For the EL characteristic, the inoculant associated with the doses of 50 and 100 kg N ha^{-1} showed an increase of 8 and 10% respectively, with the ears achieving a length of up to 14.59 cm (Table 2). At these fertiliser levels, estimates were obtained of 11.49 and 13.48 for the factor with no inoculation, while for the factor with fertiliser the estimates were 13.41 and 14.34, as per the equation in Table 3; similar to the trial carried out by Kappes *et al*. (2013), where there were increments of up to 6% in comparison with the treatment with no inoculation. It is worth noting that in the treatment with zero fertiliser but in the presence of inoculant, there was a significant increase of 37% in EL; the mean value for the ears without inoculation was 9.13 cm, increasing to 12.55 cm with the inoculant.

For the attribute ear diameter, the presence of the inoculant with zero fertiliser was sufficient for an increase in development, which corresponded to 14%. Also important was nitrogen fertiliser at 50 and 100 kg N ha^{-1} associated with the inoculant, which increased the ear diameter by 8 and 4% respectively (Table 2).

However, the greatest mean values for EL and ED were seen in the treatment with 150 kg N ha^{-1} with no inoculant, with values of 15.34 and 3.58 cm, demonstrating that in order to achieve an increase in the mean values of EL and ED the crop requires the highest dose of N used in this trial. However, satisfactory results are achieved with 100 kg N ha^{-1} associated with the diazotrophic bacterium, thereby highlighting the importance of *A. brasilense* in increasing these characteristics, since it affords increases

Table 3 - Regression equation for the attributes plant height, height of ear insertion, ear diameter, ear length, number of diseased ears, grain productivity, ear weight and 100-grain weight for the factor inoculation as a function of fertiliser level

Inoculation	Regression equation	R^2
No inoculant	$PH = 1.2504 + 0.0061x - 0.00001x^{**2}$	99.96
With inoculant	$PH = 1.562 + 0.0023x^{**}$	92.25
No inoculant	$EH = 0.579 + 0.0034x - 0.000006x^{**2}$	96.80
With inoculant	$EH = 0.711 + 0.001425x^{**}$	92.88
No inoculant	$ED = 2.75 + 0.0054x^{**}$	99.66
With inoculant	$ED = 3.158 + 0.0024x^{**}$	99.86
No inoculant	$EL = 9.52 + 0.0394x^{**}$	96.62
With inoculant	$EL = 12.485 + 0.0185x^{**}$	96.99
No inoculant	$ND = 4.25 + 0.04x - 0.00095x2 + 0.000005x^{*3}$	99.90
With inoculant	$ND = 1.4625 + 0.0417x - 0.000275x^{**2}$	53.39
No inoculant	$GP = 963.25 + 9.803x - 0.03625x^{**2}$	97.91
With inoculant	$GP = 1212.375 + 6.4975x - 0.0183x^{**2}$	93.73
No inoculant	$EW = 1123.31 + 8.73x - 0.029x^{**2}$	95.17
With inoculant	$EW = 1478.375 + 3.917x^{**}$	93.13
No inoculant	$P100 = 14.56 + 0.016x^{**}$	97.92
With inoculant	$P100 = 16.58 - 0.0078x + 0.000063x^{*2}$	71.06

X = level of fertiliser; * and ** significant at a level 5% and 1% probability by F-test in EL and ED with a reduction of 50 kg N ha^{-1}. For fertilisationwith 150 kg N, estimates of 3.52 and 3.56 cm for ear diameter were obtained for these factors withand without inoculation respectively, as per the equation in Table 3.

For GP, the treatment including inoculation with the bacterium and with zero nitrogen fertiliser, gave an increase of 25.49%, production going from 946.25 kg ha^{-1} to 1187.5 kg ha^{-1}. It should also be noted that for the remaining treatments the doses of N together with the inoculant showed significant increases. For the doses of 50, 100 and 150 kg N ha^{-1}, the increases were 10, 4 and 10% respectively. Production reached 1801.25 kg ha^{-1} for the highest dose with inoculant. At these levels of fertiliser, estimates of 1362.77, 1581.05 and 1618.07 were obtained for the factor with no inoculation, whereas for the factor with fertiliser, the estimates were 1491.50, 1679.12 and 1775.25, as per the equation in Table 3.

In relation to EW, the increases were similar to GP for the presence of bacteria and the absence of fertiliser, achieving an increase of 33.25% in production. For this characteristic, the other treatments also displayed increases of 11, 9 and 16% at doses of 50, 100 and 150 kg N ha^{-1} respectively.

The 100-grain weight showed considerable increases for only two levels of fertiliser in the presence of the *A. brasilense* bacterium;only the treatment including inoculation beingnoteworthy, corresponding to an approximate increase of 14.16% in this attribute. The second treatment that showed an increase was 50 kg N fertiliser ha^{-1} associated with the bacterium, with an increase of 7% (Table 2).

The effects found by Novakowiski *et al*. (2011) agree with the results achieved in this experiment for GP, EW and W100;those authors, irrespective of the levels of N used, obtained higher grain productivity in maize when inoculating the crop with the bacterium *A. brasilense*.

As previously pointed out, the characteristics GP, EW and W100 showed satisfactory results from inoculation with the bacteria, giving an increase in production, and reducing the costs of nitrogen fertiliser. Inoculation of ‘Iva’ landrace maizewith the diazotrophic bacteria is therefore an alternative for the rural producers of the Cariri region of Ceará for reducing the use of nitrogen fertilisers, one of the factors that most increases the costs of crop production.

For EC there was no significant difference in the AI interaction (Table 1). The estimates obtained with the inoculant did not stand out from the treatments with no inoculation (Table 2). It is believed that the increase in 100-grain weight was determinant in maintaining the levels of EC, since expansion capacity is a function of the amount of starch in the grains. By this reasoning, it can be argued that the overall increase in production did not affect grain quality.

Although low, the mean values for EC of less than 30 mL g^{-1} in the treatments both with and without inoculation with the bacterium, are not worrying at this time, since this is a landrace population in the first cycle of recurrent selection.

Technical recommendations regarding the choice of cultivar, the location, time of year and type of management applied to the crop should be followed, since increases in maize production are a result of the favourable and/or harmful influences of environmental factors (MAGALHÃES; SOUZA, 2011). It is suggested that in future research, strains should be selected that are adapted to local conditions and to the crops used in each region, especially in relation to soil and climate conditions and crop management.

CONCLUSION

Inoculation with *Azospirillum brasilense* was effective in improving all the characteristics under evaluation. Judging from the increases in NE (17.18%) and GP (10.78%), it is possible to reduce cover fertilisation to 50 kg ha^{-1} under the conditions used in the present study, without affecting crop productivity, and with a significant gain in economisingnitrogen fertiliser.

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