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### THE EFFECTS OF INOCULATION OF DIAZOTROPHIC BACTERIA ON THE INITIAL SUGARCANE GROWTH.

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#### SUMMARY

The efficiency of biological nitrogen fixation in the initial development of sugarcane, variety IACSP 95-5000, was evaluated in a greenhouse located in the Ribeirão Preto, Brazil. The trial was conducted in a completely randomized design with six replications. The plots consisted of 12L volume pots, and the soil was quartz psamments. All treatments were fertilized with 120 kg. ha<sup>-1</sup> of K<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub> and 1,15 t. ha<sup>-1</sup> of lime and N rates varied (0,60 and 120 kg.ha<sup>-1</sup>) and inoculations forms of diazotrophic bacteria, according to the following treatment: 1- Basic fertilization without nitrogen; 2. Basic fertilization with half dose of nitrogen; 3. Fertilization with normal dose of nitrogen; 4. Basic fertilization, without nitrogen with bacterial inoculation by immersion of cuttings for 1 hour; 5. Basic fertilization, without nitrogen with inoculation by bacterial inoculant sprayed. The inoculant consisted of a solution containing 10<sup>7</sup> cells per liter of solution, containing the following strains: BR11335 (Herbaspirillum seropedicae), BR11504 (Herbaspirillum rubrisubalbicans), BR11281T (Gluconacetobacter diazotrophicus), BR11366T (Burkholderia tropica) and BR11145 (Azospirillum amazonense). We evaluated the fresh and dry weight of the shoot and root, and the chlorophyll content at 60, 90 and 120 days after planting, measured at the medium portion of the leaf +3, using a portable chlorophyll meter. We concluded that inoculation by spraying provided the same fresh and dry weight of shoot and roots in relation to the levels of nitrogen applied. The chlorophyll content at120 days after the planting were equal for the doses of 60 and 120 kg ha<sup>-1</sup> of mineral-N applied in relation to forms of inoculation, either by spraying or immersion.

Keywords: Biological Nitrogen Fixation, Sugarcane, Nitrogen.

# EFEITOS DA INOCULAÇÃO DE BACTÉRIAS DIAZOTRÓFICAS NO DESENVOLVIMENTO INICIAL DE CANA-DE-AÇÚCAR.

#### RESUMO

Avaliou-se a eficiência da fixação biológica de nitrogênio no desenvolvimento inicial da cana-de-açúcar, variedade IACSP 95-5000, em casa de vegetação, localizado no Centro de Cana do IAC/APTA, Ribeirão Preto, SP. O ensaio foi conduzido em

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Delineamento Inteiramente Casualizado em 6 repetições. As parcelas foram constituídas de vasos plásticos de 12 litros, com Neossolo Quartzarênico. Foi realizada uma adubação básica, comum a todos os tratamentos, referente a 120 kg.  $ha^{-1}$  de K<sub>2</sub>O e P<sub>2</sub>O<sub>5</sub> e 1,15 t.ha<sup>-1</sup> de calcário dolomitico variando somente as doses de nitrogênio (120 e 60 kg.ha<sup>-1</sup>) e formas de inoculação das bactérias diazotróficas, conforme os tratamentos: 1. Adubação básica sem nitrogênio; 2 Adubação básica com metade da dose nitrogenada; 3. Adubação básica com dose normal de nitrogênio; 4. Adubação básica, sem adubação nitrogenada com inoculação por imersão dos toletes durante 1 hora; 5. Adubação básica, sem adubação nitrogenada, acrescida de pulverização com o inoculante. O inoculante consiste numa solução 10<sup>7</sup> células por litro de calda, contendo as estirpes: BR11335 contendo (Herbaspirillum seropedicae), BR11504 (Herbaspirillum rubrisubalbicans), BR11281T (Gluconacetobacter diazotrophicus), BR11366T (Burkholderia tropica) e BR11145 (Azospirillum amazonense). A variedade de cana utilizada foi a RB 86-7515. Foi avaliado o peso fresco e seco de raiz e parte aérea e o teor de clorofila aos 60, 90 e 120 dias plantio. Concluiu-se que a inoculação na forma de pulverização proporcionou o mesmo peso seco e fresco da parte aérea e de raízes em relação às doses de nitrogênio aplicadas. Os teores de clorofila aos 120 dias após o plantio foram iguais para as doses de 60 e 120 kg.ha<sup>-1</sup> de nitrogênio em relação às formas de inoculação, seja por pulverização ou imersão.

Palavras Chaves: Fixação Biológica de Nitrogênio, Cana-de-açúcar, Nitrogênio.

#### 1. Introduction

Sugarcane is widely cultivated in Brazil, mainly in dystrophic soils with low nitrogen contents, a critical substance for plant growth and a mandatory compound of protein and nucleic acids, participating directly or indirectly in biochemical processes and enzymatic reactions as well as a chlorophyll molecule constituent (Malavolta, 1980).

N supply to the plant depends on its availability in the soil, fertilization (soil or foliar) and biological N fixation (NBF).

Several studies have shown data indicating NBF occurrences in sugarcane, with an estimated contribution of approximately 210 kg ha<sup>-1</sup> year<sup>-1</sup> of N. A considerable number of bacteria capable of fixing atmospheric N<sub>2</sub> in sugarcane crops have been identified Urquiaga *et al.* (1992).

This study aimed to assess the effects of inoculation of diazotrophic bacteria, compared to mineral N fertilization, on initial sugarcane growth.

#### 2. Material and Methods

The experiment design was completely randomized with six repetitions. The plots consisted of 12-liter plastic vases, containing Quartz psamments, whose chemical analysis is shown in Table 1. A basic fertilization, common to all treatments, was carried out using 120 kg.  $ha^{-1}$  of K<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub> and 1.15 t. $ha^{-1}$  of lime (Basic fertilization). Only the N doses (120 and 60 kg. $ha^{-1}$ ) and inoculation methods varied, as follows: **T1.** Basic fertilization without N; **T2.** Basic fertilization with <sup>1</sup>/<sub>2</sub> N; **T3.** Basic

fertilization with total N; **T4.** Basic fertilization without N with inoculation by immersion; **T5.** Basic fertilization without N with spray solution.

The solution was prepared for immersion and spray by diluting 125 g of inoculant in 30 liters of water, resulting in a solution with 10<sup>7</sup> cells per liter of syrup, containing the following strains: BR11335 (*Herbaspirillum seropedicae*), BR11504 (*Herbaspirillum rubrisubalbicans*), BR11281T (*Gluconacetobacter diazotrophicus*), BR11366T (*Burkholderia tropica*) and BR11145 (*Azospirillum amazonense*), developed by Embrapa (Brazil).

The sugarcane cultivar used was IACSP 95-5000. We evaluated the fresh and dry weight of the shoot and root, and the chlorophyll content at 60, 90 and 120 days after planting (DAP), measured at the medium portion of the leaf +3, using a portable chlorophyll meter, model SPAD-502 Plus from Konica Minolta.

#### Table 1. Chemical and physical soil analysis.

pН	O.M	Р	K	H + Al	Al	Са	Mg	SB	CEC	V	Clay	Silt	Sand
CaCl <sub>2</sub>	g.dm <sup>3</sup>	mg.dm <sup>-3</sup>	mmol <sub>c</sub> .dm <sup>-3</sup>				%						
5,2	6	12	0,8	22	2	10	2	12,8	34,8	37	12,9	1,6	85,5
DMI ab: Soil Analysis Laboratory													

DMLab: Soil Analysis Laboratory.

#### 3. Results and discussion.

#### 3.1 – Fresh and dry weight of the root and shoot.

Spray inoculation provided similar output for fresh and dry weight compared to the N-mineral doses. Similar results were found in India in four sugarcane cultivars inoculated with a mixture of diazotrophic bacteria and mycorrhizal fungi with an equivalent response to half of the N dose recommended, Muthukumarasamy *et al.* (1999). However, some studies have observed response to inoculation, Canuto *et al.* (2003) showing that the response to inoculation is highly variable and seems to be dependent on several factors such as the plant genotype and production environment.

Immersion inoculation shows a lower output for fresh and dry weight of the shoot, when compared to the treatment with half dose of N.

Regarding fresh and dry weight of the root, we observed that spray inoculation provided the same average values when compared to inoculated N doses.

Treatments	Shoot fresh weight (g)	Roots fresh weight (g)	Shoot dry weight (g)	Roots dry weight (g)
<b>T1.</b> Basic fertilization without N	90,16 ab	25,23 ab	49,44 ab	26,14 ab
<b>T2.</b> Basic fertilization with ½	136,48 a	38,88 a	53,93 ab	27,76 ab
<b>T3.</b> Basic fertilization with total N	95,50 ab	29,78 ab	75,54 a	35,36 a
<b>T4.</b> Basic fertilization without N with inoculation by immersion	57,16 b	14,54 b	27,99 b	17,17b

## **T5.** Basic fertilization without 72,32 ab 19,61 ab 42,41 ab 26,01 ab N with spray solution

Averages followed by same small letter in the column do not differ to each other according to the Scott-Knott test at the level of 5% of probability.

#### 3.4 – Chlorophyll content

The chlorophyll content at 60 and 90 DAP was similar in the treatment with inoculation of diazotrophic bacteria when compared to doses of 60 and 120 kg.ha<sup>-1</sup> of N, except in the immersion treatment, where the chlorophyll content was lower compared to basic fertilization without N application.

However, at 120 DAP, the chlorophyll contents were equal in all treatments.

Table 5. Average values for chlorophyli content (00, 50 and 120 DAT).						
Treatments	Chlorophyll 60	Chlorophyll 90	Chlorophyll			
	DAP	DAP	120 DAP			
<b>T1.</b> Basic fertilization without N	38,73 a	41,70 a	22,16 a			
<b>T2.</b> Basic fertilization with ½ N	34,81 ab	38,60 ab	27,85 a			
<b>T3.</b> Basic fertilization with total N	35,95 ab	35,58 ab	22,23 a			
<b>T4.</b> Basic fertilization without N with inoculation by immersion	31,83 b	32,60 b	22,58 a			
<b>T5.</b> Basic fertilization without N with spray solution	35,00 ab	32,33 b	24,71 a			

Table 3. Average values for chlorophyll content (60, 90 and 120 DAP).

Averages followed by same small letter in the column do not differ to each other according to the Scott-Knott test at the level of 5% of probability.

#### 4. – Conclusion.

The inoculation by spraying provided similar values for the fresh and dry weight of the shoot and root in relation to N applied. The chlorophyll content at 120 DAP were equal for the doses of 60 and 120 kg.ha-1 of N-mineral in relation to the forms of inoculation, either spray or immersion.

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