

## Development and quality of sweet maize inoculated with diazotrophic bacteria and treated thiamine

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Received: 23/06/2018; Accepted: 12/09/2018.

### ABSTRACT

The insertion of technologies that help to maintain or increase the productivity and quality of agricultural products is highly important in the face of the challenges for the generation of more sustainable farming systems. Thus, the objective of this work was to evaluate the biometric and post-harvest characteristics of sweet maize under inoculation with *Azospirillum brasilense* and different doses of thiamine applied by seed treatment. The experiment was conducted in a factorial scheme (2 x 4) and the composition of the treatments was the combination of the presence or absence of the *Azospirillum brasilense* and different doses of thiamine (0, 15, 30 and 45 mg kg<sup>-1</sup>). Were evaluated the grain yield, ear weight with and without the straw, straw weight, straw number, ear diameter, number of grain rows per ear, number of grains per row, number of grains per ear, soluble solids, titratable acidity and ratio. When isolated, the inoculation of sweet maize seeds with the *Azospirillum brasilense* improves ears biometric and quality characteristics and the application of thiamine as seed treatment, up to the dose of 25.27 mg kg<sup>-1</sup>, improves the biometric characteristics of ears of sweet maize, however, the use of the inoculation with *Azospirillum brasilense* and the application of thiamine as seed treatment is not recommended, since the vitamin in the doses studied inhibits the bacteria action and the it potential for the biological fixation of nitrogen.

**Keywords:** *Zea mays* convar. *saccharata* var. *rugosa*, biological nitrogen fixation, vitamin B1, biostimulant effect.

### Desenvolvimento e a qualidade do milho doce inoculado com bactérias diazotróficas e tratado com tiamina

#### RESUMO

A inserção de tecnologias que auxiliem na manutenção ou aumento da produtividade e da qualidade dos produtos agrícolas possui elevada importância frente aos desafios para a geração de sistemas de cultivo mais sustentáveis. Assim, o objetivo deste trabalho foi avaliar a interação da bactéria *Azospirillum brasilense* junto a diferentes doses de tiamina via tratamento de sementes e seus efeitos na produtividade e pós-colheita do milho doce BRS Vivi. O experimento foi conduzido em esquema fatorial (2 x 4) e a composição dos tratamentos é a combinação da presença ou da ausência da bactéria *Azospirillum brasilense* e diferentes doses de tiamina (0, 15, 30 e 45 mg kg<sup>-1</sup>) aplicadas via tratamento de sementes. Observou-se que a inoculação isolada das sementes de milho doce com a bactéria *Azospirillum brasilense* melhora as características biométricas e de qualidade das espigas. Também, a tiamina aplicada isoladamente em tratamento de sementes, até a dose de 25,27 mg kg<sup>-1</sup>, melhora as características biométricas das espigas de milho doce, não sendo, no entanto, recomendada a utilização conjunta da inoculação da bactéria *Azospirillum brasilense* e a aplicação da tiamina, pois a vitamina, nas doses estudadas, inibe a ação da bactéria quanto ao potencial para a fixação biológica de nitrogênio.

**Palavras-chave:** *Zea mays* convar. *saccharata* var. *rugosa*, fixação biológica de nitrogênio, vitamina B1, efeito bioestimulante.

## 1. Introduction

Sweet maize (*Zea mays convar saccharata* var. *rugosa*) as common maize (*Zea mays* L.) crop requires large amounts of nitrogen, which is done through cover fertilizations and significantly increasing the costs of agricultural production (Kaneko et al., 2010; Leal et al., 2010). The use of diazotrophic bacteria has the potential to increase production gains, especially in systems with low nitrogen input from chemical fertilization, through the biological fixation of this nutrient (Lana et al., 2012; Dartora et al., 2013).

The nitrogen fixation is a process performed by some species of bacteria that present an enzyme called functional nitrogenase, which converts the nitrogen present in the atmosphere (N<sub>2</sub>) into forms that can be used by plants (Hungria, 2011). In the case of maize, studies involving biological nitrogen fixation are more recent and involve bacteria of the genus *Azospirillum*, which can promote plant growth and nutrient content of the grains (Longhini et al., 2016).

In addition to the techniques already used in plant production, the introduction of biostimulant compounds, such as hormones (Oliveira et al., 2016), algae (Ribeiro et al., 2017) and, recently, vitamins (Vendruscolo et al., 2017; Vendruscolo et al., 2018), which stimulate the development of the plants and the monetary gains obtained by their sale, is observed (Jesus et al., 2016). In this sense, studies demonstrate that vitamins can be used to improve the vegetative and reproductive development of species of economic interest, among which are those vitamins belonging to the B complex (Hendawy and El-Din, 2010; Mohsen et al., 2013; Soltani et al., 2014).

Thiamine, or vitamin B1, is present in many foods, including cereals like maize. It activates the plant's defense mechanisms against biotic and abiotic stresses, improving water and nutrient uptake by increasing nutrient and energy reserves (Ahn et al., 2005; Boubakri et al., 2012; Kaya et al., 2015). These effects culminate in gains in the vegetative and reproductive development of different plant species (Oertli, 1987; Hendawy and El-Din, 2010; Soltani et al., 2014). Despite the benefits of introducing vitamins into the production system of some crops, the action of these elements as activators of resistance mechanisms can be an obstacle to the use of biological nitrogen fixation due to the antagonistic action, thus justifying the generation of information about this interaction.

The objective of this work was to evaluate the biometric and post-harvest characteristics of sweet maize under inoculation with *Azospirillum brasilense* and different doses of thiamine as seed treatment.

## 2. Material and Methods

The study was conducted in the experimental area of the Agronomy School, at the Federal University of Goiás, located in the city of Goiânia, Goiás State, Brazil. The municipality is in the Center-West region under latitude 16°40'S, longitude 49°15'W and altitude of 750 m. This region has an Aw climate, according to KÖPPEN-GEIGER classification (Cardoso et al., 2014) characterized by a tropical climate with a rainy season from October to April and a period with precipitations below 100 mm monthly from May to September. The mean monthly temperatures vary from 20.8°C in June and July to 25.3°C in October (Cardoso et al., 2014).

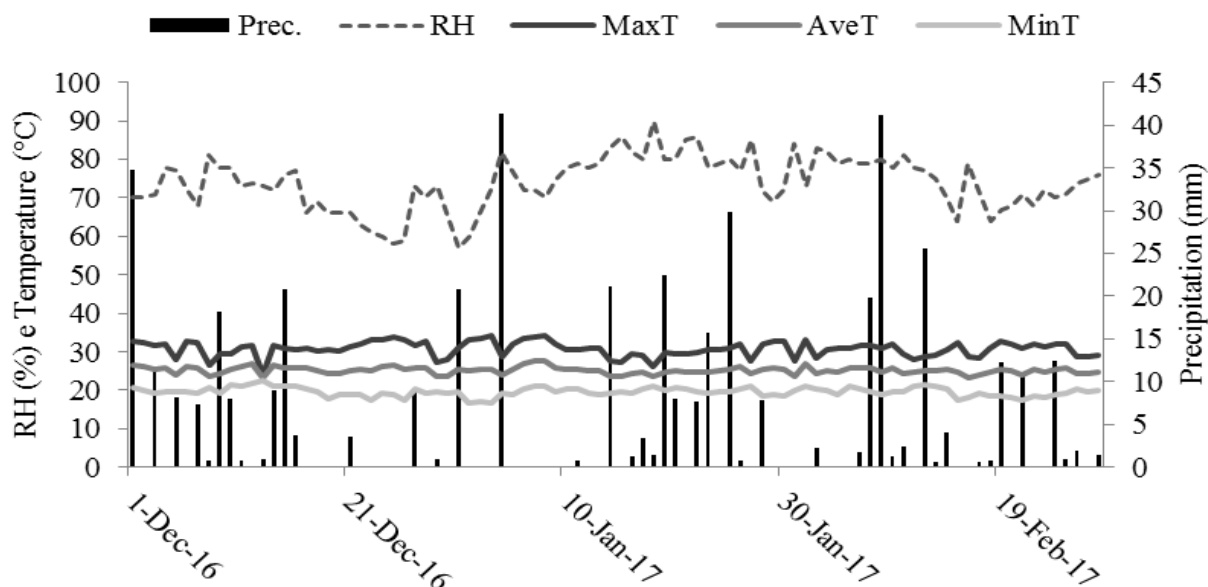
The temperature, relative air humidity and precipitation data were recorded during the conduction of the experiment from daily readings performed at the evaporimetric station, located 100 m from the experimental area (Figure 1).

Prior to planting, the desiccation of the spontaneous plants with herbicide based on glyphosate (48% i.e.) was carried out. The soil present in the experimental area was classified as LATOSOLO VERMELHO (Santos et al., 2013) and through laboratory analysis showed the following characteristics: Ca<sup>2+</sup>: 3,5 cmol<sub>c</sub> dm<sup>-3</sup>, Mg<sup>2+</sup>: 1,80 cmol<sub>c</sub> dm<sup>-3</sup>, K<sup>+</sup>: 120,0 mg dm<sup>-3</sup>, P (Mehlich I): 7 mg dm<sup>-3</sup>, Organic matter: 16 g kg<sup>-3</sup>, Al<sup>3+</sup>: 0,0 cmol<sub>c</sub> dm<sup>-3</sup>, H+Al: 1,1 cmol<sub>c</sub> dm<sup>-3</sup>, pH (CaCl<sub>2</sub>): 6,4, CTC: 6.71 cmol<sub>c</sub> dm<sup>-3</sup>, V: 84% (Donagemma et al., 2011). The granulometric analysis of the soil presented 450 g kg<sup>-1</sup> of clay in the 0-0.2 m layer (Silva, 2009).

The experiment was conducted in a randomized block design, in a factorial 2 x 4 and four repetitions. The treatment composition was the combination of the presence or absence of the bacterium *Azospirillum brasilense* (NITRO 1000 Gramíneas, NITRO 1000, Cascavél, PR, Brazil), in dosage of 100 ml ha<sup>-1</sup> and different doses of thiamine (Neon, Suzano, SP, Brazil), 0.00, 15.00, 30.00 and 45.00 mg kg<sup>-1</sup> applied as seed treatment. The bacteria and the vitamin doses were applied directly to the seeds, packed in transparent polyethylene bags and vigorously stirred for 1 min for the homogeneous products distribution.

Each experimental plot consisted of an area of 10.00 m<sup>2</sup>, five lines of 4.00 m in length, in four replications. The planting was carried out on December 1, 2016, spacing 0.50 m between rows and 0.30 m between plants, generating an average population of 66,700 plants ha<sup>-1</sup>.

During the planting, no soil fertilization was performed due to the organic matter, phosphorus and potassium levels present in the area. N and K fertilization were performed with urea (45% N) 25 kg ha<sup>-1</sup> and KCl 40 kg ha<sup>-1</sup>, respectively, at the V2/V3 stage. 45 days after sowing a second urea cover 25 kg ha<sup>-1</sup> was performed at V4/V5 stage of the plants development.



**Figure 1.** Climatic conditions of maximum, average and minimum temperature, relative air humidity and precipitations during the experimental period.

One application of LAMBDA-CIALOTRIN (50% a.i.) insecticide was carried out to control *Spodoptera frugiperda*, 45 days after sowing, in V4 stage, at the dose of 150 ml ha<sup>-1</sup>. Harvesting of the spikes with straw was accomplished 80 days after sowing, when the grains presented 75% of moisture, for the evaluation of the variables of productivity and post-harvest.

Grain yield was estimated by multiplying the mass of grains per ear by the population of plants in one hectare. The ear weight with and without the straw and the straw weight were obtained in semi-analytical balance (ML 600, Marte, São Paulo, SP, Brazil). The straw number, number of grain rows, number of grains per row and number of grains per ear were counted and the ear diameter was obtained with a digital caliper (Metrotools, São Paulo, SP, Brazil).

In the post-harvest evaluations were analyzed the soluble solids, where the °Brix were sampled in an analogic manual refractometer (RTA-50, Instrutherm, São Paulo, São Paulo, Brazil). The titratable acidity was determined by titration with 0.1 N sodium hydroxide solution (NaOH). The ratio between soluble solids and titratable acidity (SS/AT), known as *ratio*, was also calculated. The pH was obtained with a bench pH meter (PHB-500, Ionlab, Araucária, Paraná, Brazil).

The data were analyzed through analysis of variance and Tukey test at 5% of probability for the qualitative factor of inoculations with *A. brasilense* and regression analysis for the quantitative factor of thiamine doses. For the data analyzes was used the computer program System for Variance Analysis – SISVAR (Ferreira, 2014).

### 3. Results and Discussion

Exploring separately the *A. brasilense* inoculation factor, it was verified a significant difference only for the straw mass. For this variable, the inoculation resulted in a 10.82% decrease in the straw mass compared to the non-inoculated plants. The number of grain strains per row, number of grains per spike and grain yield were not affected by the treatments used (Table 1).

The interaction of the treatments *A. brasilense* x thiamine in respect of the variable weight of the ear with the straw was observed (Figure 2). The inoculation with the bacteria provided a significant increase for this variable, in the order of 22.72%, when compared to the control without inoculation and without application of thiamine.

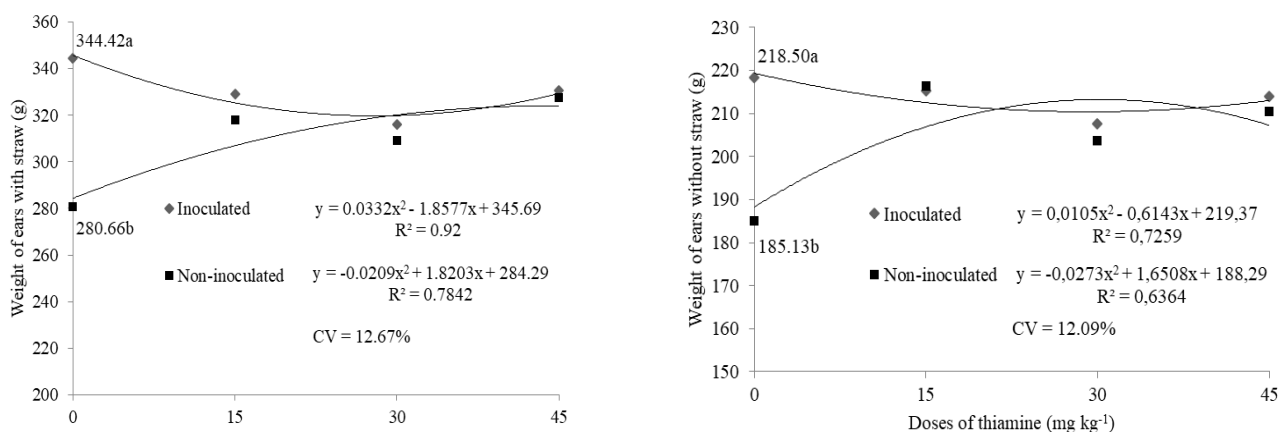
The negative effect of the combined application was observed up to the maximum dose of 28.01 mg kg<sup>-1</sup>, for which a loss of 24.75 g per ear was observed. Nevertheless, the isolated application of thiamine was beneficial for the development of the ears. It was observed that there was an increase of up to 43.23 g per ear when increasing doses were used up to 43.51 mg kg<sup>-1</sup>.

The positive effect observed in relation to the *A. brasilense* inoculation is related to the nitrogenase enzyme, that is able to break the triple bond between two molecules of N<sub>2</sub>, reducing the ammonia, the same form obtained from the industrial process, making available to the plant part of this broken nitrogen (Hungria, 2011). According to Lodewyckx et al. (2002), more than 80 genres of endophytic bacteria have been found colonizing the interior of the tissues of plants, among them the genre *Azospirillum*.

**Table 1.** Weight of ears straw (WS), straw number per ear (SN), ear length (EL), number of grain rows per ear (NRE), number of grains per row (NGR), number of grains per ear (NGE) e grain yield (GY) of sweet maize plants inoculated or not with *A. brasilense* and different doses of thiamine.

<i>A. brasilense</i>	WS (g)	SN -	EL (cm)	NRE -	NGR -	NGE -	GY (Mg ha <sup>-1</sup> )
Inoculated	104.84b*	10.20a	16.92a	15.15a	37.23a	563.28a	6.33a
Non-inoculated	116.18a	10.48a	17.29 a	14.70a	37.43a	548.5a	6.76a
MSD	8.69	0.45	0.43	0.58	1.14	24.20	0.59
Thiamine (mg kg <sup>-1</sup> )							
0	110.75	10.57	17.02	14.60	37.13	539.27	6.33
15	107.59	9.87	17.24	14.87	38.13	565.20	6.79
30	106.90	10.43	16.90	15.27	37.13	566.40	6.40
45	116.80	10.50	17.23	14.97	36.93	552.70	6.67
LR	Ns	Ns	Ns	Ns	Ns	Ns	Ns
QR	Ns	Ns	Ns	Ns	Ns	Ns	Ns
CV %	21.74	12.08	6.94	10.78	8.47	12.03	24.99

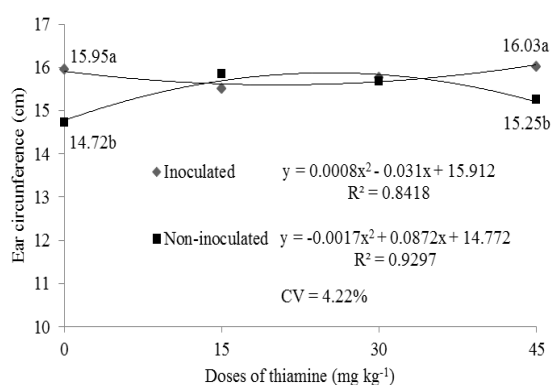
\*Means followed by the same letters are not significant at  $p < 0.05$  by the Tukey test. MSD = minimum significant difference; LR = linear regression; QR = quadratic regression; CV = coefficient of variation.

**Figure 2.** Weight of ears with and without straw of sweet maize plants inoculated or not with *A. brasilense* and treated with different doses of thiamine.

The action of the bacteria is related to the atmospheric nitrogen fixation and to the best use of the nitrogen added to the system through fertilization (Morais et al., 2015). This nutrient has an essential role in the development and functioning of plant tissues, being indispensable to the photosynthetic system and, consequently, to the carbon fixation and construction of energy reserves of plants (Taiz et al., 2017).

In addition to the nitrogen partition, *A. brasilense* produces hormones that stimulate root growth, the main one being auxin, indoleacetic acid (AIA) (Crozier et al., 1988). By stimulating root growth it promotes higher absorption of water and nutrients, also favoring aerial growth and increased dry matter production, resulting in productivity increases.

For the variable ear circumference, similar behavior to other biometric variables was observed for differences between inoculated and non-inoculated plants (Figure 3), without application of vitamins, where inoculation provided a significant increase of 8.33%. Again, the application of thiamine provided a decrease in the values of the variable, when inoculation with the bacterium, up to the dose of 20.32 mg kg<sup>-1</sup>, and an increase of the diameter when the application of the vitamin was carried out of isolated form until 25.27 mg kg<sup>-1</sup>. At these points, the reduction and the increase of the diameters were of 2.24% and 7.84%, respectively, in relation to the treatments without the application of thiamine.



**Figure 3.** Ear circumference of sweet maize plants inoculated or not with *A. brasilense* and treated with different doses of thiamine.

When the application of thiamine was carried out, there was inhibition of the beneficial effect of the bacteria. This result is related to the activation of the plant defense mechanisms when it is treated with the vitamin (Ahn et al., 2005; Boubakri et al., 2012). The thiamine is an activator of resistance in plants against many fungal, bacterial and viral infections, besides acting as a coenzyme in several metabolic processes (Oertli, 1987; Ahn et al., 2005; Boubakri et al., 2012). The resistance induction process occurs because thiamine stimulates the resistance genes to perceive the virulent agent more quickly and efficiently, thus providing cell death at the site of infection, eliminating the infected cells and preventing the pathogen from contaminating the entire plant (Ahn et al., 2005).

For the post-harvester characteristics, it was observed a significant difference only for titratable acidity (TA), which was significantly higher in spikes from plants non-inoculated with *A. brasilense* (Table 2).

For the ratio (SS/AT), found in this study, was observed a mean of 39.44 for treatments without *A. brasilense* inoculation and 36.93 for treatments with

inoculation. It was verified that the sweet maize used in the present study has superior results when compared to other commercial hybrids, and even when compared to BRS Vivi hybrid cultivated under different conditions (Teixeira et al., 2014; Pinho et al., 2008).

For the titratable acidity was observed that the values obtained in the present study were lower than those obtained in other studies, including with the same cultivar (Teixeira et al., 2014; Pinho et al., 2008). The negative effect of the inoculation is related to the nitrogen capacity to increase the ears size. It was also observed for onions (Rodrigues et al., 2015) and for pineapples (Silva et al., 2015), both cultivated with different doses of nitrogen. The authors verified that higher doses of nitrogen increased the fruit size but it diluted the titratable acidity.

The soluble solids content are important compounds responsible for the flavor, the most important being the sugars and organic acids (Lima, 2007), and in the present study, there was no significant difference between the treatments. However, as mentioned above for the other characteristics, in this experiment the averages were higher when compared with other commercial hybrids and the BRS Vivi cultivated under different conditions (Teixeira et al., 2014; Pinho et al., 2008).

The results obtained on this study showed that both, the *A. brasilense* and the thiamine, have the potential to stimulate the sweet maize production. However, more studies are needed for the comprehension of the effects on different commercial species or even on different genotypes. Nevertheless, the ears quality promoted by the thiamine application also showed that this vitamin could be included as a biostimulant in the commercial agriculture, improving the gains for the producers.

**Table 2.** Grain titratable acidity, soluble solids and pH of sweet maize plants inoculated or not with *A. brasilense* and treated with different doses of thiamine.

<i>A. brasilense</i>	Titratable acidity Citric acid g 100g <sup>-1</sup>	Soluble solids °Brix	pH
Inoculated	0.41b <sup>*</sup>	16.17a	6.91a
Non-inoculated	0.44a	16.25a	6.93a
MSD	0.02	0.16	0.07
Thiamine (mg kg <sup>-1</sup> )			
0	0.42	16.17	6.91
15	0.44	16.50	6.94
30	0.42	16.17	6.91
45	0.41	16.00	6.94
LR	Ns	Ns	Ns
QR	Ns	Ns	Ns
CV %	11.03	2.07	2.03

\*Means followed by the same letters are not significant at  $p < 0.05$  by the Tukey test. MSD = minimum significant difference; LR = linear regression; QR = quadratic regression; CV = coefficient of variation.

#### 4. Conclusions

When isolated, the inoculation of sweet maize seeds with the *A. brasilense* improves ears biometric and quality characteristics and the application of thiamine as a seed treatment, up to the dose of 25.27 mg kg<sup>-1</sup>, improves the biometric characteristics of ears of sweet maize. However, the use of the combined inoculation with *A. brasilense* and application of thiamine as seed treatment is not recommended. Since the vitamin in the doses studied inhibits the bacteria action and the potential for the biological fixation of nitrogen.

#### Acknowledgements

We would like to thank the Coordination of Improvement of Higher Level Personnel - CAPES for granting the scholarship to the first author and Embrapa Milho e Sorgo for the donation of sweet maize seeds, cultivar BRS Vivi.

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