

ARBUSCULAR MYCORRHIZAS IN SUGARCANE UNDER DIFFERENT PLANTING SYSTEMS AND SOURCES OF NITROGEN

Micorrizas arbusculares em variedades da cana-de-açúcar sob diferentes sistemas de plantio e fontes de nitrogênio

Jadson Belem de Moura^{1*}; Diego André Ribeiro²; Luiz César Lopes Filho³; Rodrigo Fernandes de Souza⁴; Leonnardo Cruvinel Furquim⁵

¹Doutor em Agronomia; Faculdade Evangélica de Goianésia; email: jadsonbelem@gmail.com

*Autor para correspondência

²Bacharel em Agronomia; Faculdade Evangélica de Goianésia; diegoandreribeiro@hotmail.com

³Mestre em Ciências Agrárias; Instituto Federal Goiano - Campus Rio Verde – GO; Brasil. lopesfilholuizcesar@gmail.com

⁴Mestre em Agronomia; Faculdade Evangélica de Goianésia; rodrigoofunb@gmail.com

⁵Doutorando em Agronomia; Faculdade Objetivo; leonnardo.cruvinel@faculdadeobjetivo.com.br

Artigo enviado em 17/02/2017, aceito em 02/07/2017 e publicado em 29/09/2017.

Abstract This study aimed to evaluate the density of spores and mycorrhizal colonization in roots of sugarcane varieties (RB86-7515, CTC-4 and CTC-15), under planting systems, being the planting of stem portions and pre-sprouted seedlings (PSS) along with 3 sources of nitrogen: granular fertilizer, leaf application and inoculation with diazotrophic bacteria (*Azospirillum brasilense*). The experiment was deployed on the experimental campus of the Evangelical School of Goianésia, and was collected soil samples for spore count after the first cut. The experimental design was arranged in factorial 3x2x3 in randomized blocks with subdivided plots using 4 repetitions per treatment, where factor 1 was represented by varieties, factor 2 by planting systems and factor 3 by nitrogen sources. The planting of PSS associated with foliar fertilization and *A. brasilense* presented better colonization than in PSS with granular fertilizer and stem portions with leaf fertilizer and via inoculant. The granular fertilizer showed increased amounts of spores over the foliar fertilization and inoculation of *Azospirillum brasilense*, the planting system of stem portions presented greater quantity of spores over the planting of PSS, it is worth mentioning that approximate results of spore density, mycorrhizal colonization and productivity without adding granular fertilizer are satisfactory for reducing the use of fertilizers.

Keywords - Pre-sprouted seedlings, biological nitrogen fixation, mycorrhizal fungi, *Azospirillum brasilense*.

Resumo – Este estudo objetivou avaliar a densidade de esporos e colonização micorrízica em raízes de diferentes variedades de cana-de-açúcar (RB86-7515, CTC-4 e CTC-15), sob diferentes sistemas de plantio, sendo plantio de toletes e de mudas pré-brotadas (MPB) e 3 diferentes fontes de nitrogênio a adubação granular, adubação foliar e inoculação com bactéria diazotrófica (*Azospirillum brasilense*). O experimento foi implantado no campus experimental da Faculdade Evangélica de Goianésia, e coletadas amostras de solo para contagem de esporos após o primeiro corte. O delineamento experimental adotado foi um fatorial 3x2x3 em blocos casualizados com parcelas sub-subdivididas, utilizando 4 repetições por tratamento, onde o fator 1 representado por variedades, fator 2 por sistemas de plantio e fator 3 por diferentes fontes de nitrogênio. A adubação granular apresentou maiores quantidades de esporos sobre a adubação foliar e inoculação de *Azospirillum brasilense*, no sistema de plantio de toletes foi observado maior quantidade de esporos que no plantio de MPB, o plantio de MPB associado a adubação foliar e *A. brasilense* apresentaram melhores médias de colonização que no MPB com adubação granular e do plantio em tolete com adubação foliar e via inoculante, vale ressaltar que resultados aproximados de densidade de esporos, colonização micorrízica e produtividade, sem adição de adubação granular são satisfatórios por reduzir o uso de fertilizantes.

Palavras-chave - Mudas pré-brotadas, fixação biológica de nitrogênio, fungos micorrízicos, *Azospirillum brasilense*.

INTRODUCTION

The sugarcane culture (*Saccharum spp.*) plays a very important role in the world economy (COELHO et al., 2003; GARVEY; BARRETO, 2014; VIEIRA, 2010). Brazil is currently the largest producer of the culture of sugar and ethanol both come from sugarcane. This country represents more than 50 % of sugar of the world's sugar marketed (CONAB, [s.d.]).

The sugarcane planting is usually done by portions of stem, containing one or more buds (LANDELL, 2012; SILVA; JERONIMO; LÚCIO, 2008). Landell (2012) explains that over a period of approximately 60 days the stem portions reserves is the key to the sprouting and it reduces dependency relationship with the development of the root system and aerial parts, increasing the ability to absorb water and nutrients from the soil and making the autotrophic plant. The quality of the sprouts determines the percentage of survival, growth rate of tiller and final production. In addition, sprouts of good quality have greater growth potential, exerting better control of invasive plants, therefore, reducing costs with cultural practices (MORGADO et al., 2000; SILVA; JERONIMO; LÚCIO, 2008).

The different way to spread the sugarcane is the planting of pre-sprouted seedlings (PSS). This technology allows us to change the shape of seedling production. Instead of using the pieces of stem for propagating, pre-sprouted seedlings are inserted, which come from the “minirrebolos” (stem parts with buds). This technology may contribute to the fast production of seedlings, increasing pattern of plant health, vigour and uniformity, in addition to reducing the amount of sprouts going to the field. To plant one hectare (ha) of sugarcane, seedling consumption varies from 18 to 20 tons in conventional planting, with PSS it is dropped to 2 tons (LANDELL, 2012). After approximately 60 days from the beginning of the production process, the sprouts must be in ideal conditions to be sown in the field, but it is expected that the seedlings are in a good vigour and well rooted. This process preserves the integrity of the root system, minimizes problems with water stress and facilitates the planting and rooting of sprouts (LANDELL, 2012; TRUBER; FERNANDES, 2014).

Although nitrogen only contributes with 1 %, on average, in the dry matter, the annual requirement of sugarcane is on the order of 100 kg/ha. Its role is as important as the carbon, hydrogen and oxygen, which together constitute more than 90 % of dry matter (VITTI et al., 2007). Hungria (2011) says that Brazil imports 73 % of nitrogen used in agriculture, which increases substantially the costs of fertilization.

Diazotrophic bacteria can associate with the sugarcane naturally or can be inoculated to promote

beneficial effects (GÍRIO et al., 2015; SCHULTZ et al., 2012). In line with Hungria et al. (2016) several studies have been carried out trying to identify micro-organisms that make symbiosis with grasses, providing N, with the identification of some genres, such as the *Azospirillum*.

As an alternative to an increase in the absorption of N, the arbuscular mycorrhizal fungi (AMFs) form a symbiotic relationship with the plant, the result is to increase the contact surface with the ground root system promoted by the mycelium formed externally to the roots. This increases photosynthetic capacity, production of growth regulatory substances and enzymatic activity, resulting in increased capacity of plant development. These metabolic changes provide greater resistance to pests, diseases, water shortages, nutritional deficiencies and thermal stresses. Ecologically, mycorrhizal association enables better utilization and conservation of nutrients available in the soil-plant system, as well as the greater adaptability of seedlings transplanted. The effects of mycorrhizal association are attributed mainly to the extra radicular development of the mycelium, which happens after the colonization of roots by the fungus. (FILHO; NOGUEIRA, 2007; GAI et al., 2015; MOHAN et al., 2014; MOURA, 2015; SILVEIRA; FREITAS, 2007; SOUZA et al., 2016).

Barbara et al. (2006), the fungi in addition to the nutrition of plants, contributes to the structuring of the soil and plant communities. Jastrow et al. (1998) reported that the immense amount of hyphae produced by AMF influences on the structure and stability of aggregates in soils. Thus, the present study aimed to evaluate the density of spores in the soil and mycorrhizal colonization rate, productivity, brix, fiber and TRS in sugarcane varieties under plantation systems and sources of nitrogen.

MATERIAL AND METHODS

The experiment was deployed on the experimental campus of the Evangelical School of the Goianésia Town, situated at coordinates 15° 19 ' 22.32 " S and 49° 08 ' 19.85 " W in Goianésia, Goiás. According to Köppen (1931) the climate is classified as seasonal tropical (Aw), being characterized by two well defined seasons (dry and rainy season), as well as the occurrence of periods of drought during the rainy season. The experimental design consisted of a factorial design 3x2x3 in randomized blocks with subdivided plots using 4 repetitions per treatment, where factor 1 was represented by varieties, factor 2 by planting systems and factor 3 by nitrogen sources. For the implementation of the experiment, we collected soil samples classified as dystrophic Red Latosol. The samples were sent to the laboratory for analysis of soil at Jalles Machado Power Plant S/A. The results are shown in table 1.

Table 1. Soil analysis at 0 - 20 cm depth in the experimental campus of the Evangelical School of Goianésia,

Production System	Depth	pH CaCl ₂	P Mgdm ⁻³	K -----Mmolc-----	Ca	Mg	H+Al	MO g/kg-3	Sand	Silt	Clay
										-----g.kg ⁻¹ -----	
Conventional	0-20	5.9	3.4	0.108	1.7	1.01	2	21.5	-	-	24.1

After the sampling study began the soil was prepared with plowing and harrowing. The planting was held on 23 August 2014, divided into 6 rows of 8 meters long, spaced at 1.5 m. Granular fertilizer was applied with 6/30/10 formulation in a ratio of 600 kg/ha and during the planting at a ratio of 14 Ton/ha. The planting adopted two methods, stem portions by distributing 15 buds/linear metre and pre-sprouted seedlings method (PSS) transplanted seedlings at 3/linear metre. The sprinkler irrigation system was used, with a 60 mm blade in pre-planting irrigation, and a 120 mm blade in irrigation of post-planting divided into two 60 mm blades.

The sugarcane varieties used were RB86-7515, CTC-15 and CTC-04, all cultivars with great emphasis on productivity in the region and were cultivated by the Usina Jalles Machado S/A.

Fertilization was conducted with two different sources of nitrogen, the first fertilization was into 60 days after planting and another fertilization at 180 days after planting. The sources used were Biozyme (20L ha⁻¹ / 400g of N ha⁻¹), *Azospirillum brasilense* (20L ha⁻¹ / 5 x 10⁸ cells / mL) and the granular fertilizer 10-00-40, (400kg ha⁻¹), all applied manually. These doses were applied throughout the whole experiment. We used the commercial inoculant Nitro1000, composed of *Bradyrhizobium japonicum*, Semia 5079 and Semia 5080, vitamins, minerals, carbon source, peat (powder) and water, thickener, preservative and stabilizer PVP (aqueous) of Nitro1000®

On August 29th, the harvest was performed manually and the samples were transported to the Chemistry Laboratory of the Jalles Machado Sugarcane Power Plant, where we made the fruit sugar concentration (Brix), TRS (Total Recovered Sugar), fiber and productivity.

Roots and rhizospheric soil were collected in all treatments. The samples were collected from 0 to 20 cm depth within 1 m² for each parcel in the spaces which occurred the harvesting of sugarcane. The samples collected were placed into plastic bags and stored with room temperature until the moment of the extraction of spores.

Spores were extracted from soil using 50 cm³ of each composite sample, by wet sieving technique

(GERDEMANN; NICOLSON, 1963) followed by centrifugation in sucrose solution and water up to 50 %. The spores were separated according to their phenotypic characteristics such as color, size and shape, composing the different morphotypes, under stereoscopic binocular magnifying glass.

For the determination of the percentage of fungi colonization, we observed the mycorrhizae contained within the root cortex using thin roots collected in the soil samples. Roots were submitted at first to a wash in 10 % KOH solution in 1 litre, heated to 90° C in heating blanket for 60 minutes, then held in washing 1 % HCL solution in 1 litre, also heated to 90° C in heating blanket for 60 minutes, followed by the step of coloring, using the composition of 750 ml of acetic acid and 40 ml of ink for blue stamping, heated to 90° C in manta for 10 minutes (PHILLIPS; HAYMAN, 1970).

Each sample was stored in containers with lactoglycerol solution (glycerol, lactic acid, distilled water), then kept in the refrigerator until the count. The evaluation of colonization was did in stereoscopic microscope, following the technique of intersection of the quadrants (GIOVANNETTI; MOSSE, 1980).

For statistical analysis it was used the ASSISTAT 7.7 statistical program beta (SILVA; DUARTE; CAVALCANTI-MATA, 2010) applying the Scott Knott test at 5 % probability.

RESULTS AND DISCUSSION

The variance analysis (table 2) shows the evaluation of the spore density with significant differences when compared to the different varieties, planting systems and nitrogen sources. Thus, there was no significant difference regarding the interactions evaluated among the variables tested. Regarding mycorrhizal colonization the F test showed significant difference only in the interaction between planting system and nitrogen sources. However, there was no significant difference in other variables evaluated.

Table 2. F-test of arbuscular mycorrhizal in sugarcane varieties under different planting systems and sources of nitrogen.

Treatment	Spore Density	Mycorrhizal colonization	Productivity	Brix	Fiber	TRS
Variety	9.88 *	0.31 ns	1.21 Ns	1.56 ns	6.71*	20.05*
Planting System	6.90 *	2.43 ns	46.22*	10.55*	0.65 ns	1.73 ns
Sources of Nitrogen	3.10 *	0.83 ns	1.64 Ns	9.19*	0.75 ns	1.77 ns
Variety x Planting System	0.52 ns	1.00 ns	16.15*	13.70*	0.85 ns	12.98*
Variety x Source of Nitrogen	0.64 ns	2.31 ns	1.50 Ns	11.06*	0.16 ns	1.30 ns
Planting System x Source of Nitrogen	1.07 ns	3.28*	1.20 Ns	11.72*	0.14 ns	0.18 ns

* - significant at probability level 5 % (01 =< p < .05)

ns- not significant (p >= .05)

There was minimum significant difference in spore density values, fiber content and TRS, in varieties RB86-7515, CTC4 and CTC15 (Table 3).

Table 3. Spore density (n° of spores/50cm³), Mycorrhizal colonization rate (%), Productivity (Mg/ha), Brix (Kg/ha), Fiber (Kg/ha) and TRS (kg/ha) of three of sugarcane varieties.

Variety	Spore Density	Mycorrhizal colonization	Productivity	Brix	Fiber	TRS
	n° of spores/50cm ³	%	Mg/ha	-----Kg/ha -----		
RB86-7515	129.16 a*	20.33 a	126.33 a	2116 a	1347 b	14843 b
CTC4	95.58 a	20.00 a	151.41 a	2091 a	1335 b	14876 b
CTC15	48.58 b	21.08 a	137.50 a	2190 a	1466 a	16698 a

* Medium numbers followed by the same letter do not differ statistically between themselves. The Scott-Knott test was applied at 5 % probability.

The varieties RB86-7515 and CTC4 presented higher average density of spores when compared to the variety CTC15. Gírio et al. (2015) describes the variety RB86-7515 as highly responsive to inoculation with growth-promoting bacteria. The study by Reis et al. (1999) using 14 different varieties and 3 samples collection sites on sugarcane, they found spore number ranging from 18 to 100 mL of 2,070 for soil, and average 38 to 1,630 for 100 mL of soil. The plant genotype is able to determine the interaction between bacteria and plant, and the efficiency of the NFB (BODDEY et al., 2003; OLIVER, 2014; URQUIAGA; CRUZ; BODDEY, 1992). RB72-454 and RB86-7515 varieties were inoculated with polymers and presented 13 % and 20 % increase respectively in nitrogen for the plants (SILVA et al., 2009).

Similar results were found by Rao (2012) for the fiber content, the best averages were obtained for varieties CTC-15, RB93-5744 and SP80-3280.

Conforming to Freitas et al. (2006), the main factors related to genetic quality are plants with a high sucrose

content, purity and percentage of fiber in sugarcane. The main sugarcane varieties are the ones that have high sucrose content and resistance to pests and diseases.

The sugarcane presents features of great interest to cattle ranchers, as high dry matter production per hectare, easy handling and persistence of culture, good acceptance by animals, high content of soluble carbohydrates and lower cost of production. Besides sucrose content, the quality of the fiber is the fundamental importance when sugarcane is supplied to ruminants, demonstrating the need for the establishment of selection criteria aiming specifically to animal feeding (FREITAS et al., 2006).

The Total Sugar Recovered (TRS) is very important for both the industry and for producers. Conforming to the referenced variable is that industrial units work out the price paid to producers, following a methodology described by CONSECANA (2006).

Significant differences in spore count, productivity and brix in stem portions and pre-sprouted seedlings plantings were found (Table 4).

Table 4. Spore density (n° of spores/50cm³), Mycorrhizal colonization rate (%), Productivity (Mg/ha), Brix (Kg/ha), Fiber (Kg/ha) and TRS (kg/ha) of two planting systems.

Planting System	Spore Density	Mycorrhizal colonization	Productivity	Brix	Fiber	TRS
	n° of spores/50cm ³	%	Mg/ha	-----Kg/ha -----		
PSS	76.97 b*	21.27 a	119.08 b	2049 b	1371 a	15195 a
Stem Portion	105.25 a	19.88 a	157.75 a	2216 a	1394 a	15749 a

* Medium numbers followed by the same letter do not differ statistically between themselves. The Scott-Knott test was applied at 5 % probability.

The planting system of stem portions presented the highest amount in number of spores when compared to the planting of PSS. This result may be due to increased nutrient reserves of the stem piece, as quoted by Carneiro et al. (1995), and greater volume of roots, comparing Gírio et al. (2015) research mentioned that in the initial phase the plant requires a large part of the nutrients contained in the stem portions. These works attest to the relation between root volume and the amount of spores. Related to the fact the bacteria it can easily penetrate in the roots promoting greater interaction between fungus and plant (GOMES et al., 2005; SOUZA et al., 2016).

Pre-sprouted seedlings (PSS) of sugarcane is a multiplication system that could contribute to the rapid production of seedlings, associated with a high standard of

plant health, vigour and uniformity of planting (LANDELL, 2012).

Gírio et al. (2015), He concluded in his study that the use of pre-sprouted seedlings increases the speed of sprouting, the accumulation of dry matter in the roots and productivity regardless of the amount of the reserve. The energy reserved accumulated at the base of the stalk, favours the germination of sugarcane plantations, responding with increased tillering and productivity (SILVA; JERONIMO; LÚCIO, 2008).

Carneiro et al. (1995) had similar results when he demonstrates that the stem portions directly influences the early development of plant canopies, and it should be a factor, along with several others, to influence the productivity of the sugarcane plant. Two of the systems

evaluated, PSS and stem portions, the response of the harvest of sugarcane from the stem portions, obtained better results of ° brix correlating with the seedlings of PSS. The soluble solids (brix in %) is the percentage in grams of dissolved solids in the present water in a product (sugarcane), the determination of brix is made from the liquid extracted from sugarcane.

The brix reading is held usually to determine the stage of maturation of culture, establishing a correlation between the solid content of the base and the apex. (PARANHOS, 1987).

Brix values increase during ripening, as the age of the plant and the climatic conditions become favourable, the

varieties ripen differently during the period, some of them have higher brix values at the beginning of the ripening, while others in the middle or at the end of maturation.

The biggest differences between varieties are found by the genotype and at the beginning of maturation. The brix is significantly influenced by variety, by periods of sampling and their interactions. Resulting in greater productivity of sugarcane when planted in stem portions, in agreement with table 5, there was significant difference in all varieties planted in stem portions with higher productivity than the PSS. (TANEJA et al, 1986).

Table 5. Productivity (Mg,ha⁻¹) of different of sugarcane varieties under planting systems: stem portions and PSS.

Varieties	Planting System	
	PSS	Stem Portions
RB868-7515	129.83 aA*	122.83 bA
CTC-4	121.75 aB	181.08 aA
CTC-15	105.66 aB	169.83 aA

* Medium numbers followed by the same letter do not differ statistically between themselves. The Scott-Knott test was applied at 5 % probability.

The works of Gomes et al. (2005) he points out that diazotrophic bacteria of various genres, among them those belonging to the genre *Azospirillum* can be inserted naturally in the stem portions used for the propagation of sugarcane.

When compared to different sources of nitrogen in the evaluation of the density of spores, there was significant difference as shown in table 6. The granular fertilizer showed the highest volume of spores on the foliar fertilization and inoculation by bacteria.

Table 6. Spore density (n° of spores / 50g of soil) of sugarcane under different sources of nitrogen.

Source of Nitrogen	Spore Density	Mycorrhizal colonization	Productivity	Brix	Fiber	TRS
	n° of spores/50cm ³	%	Mg/ha	-----Kg/ha -----		
Granular Fertilizer	110.83 a*	20.001	131.37 a	2166 a	1412 a	15123 a
Leaf Fertilizer	81.66 b	21.00	136.16 a	2036 b	1357 a	15676 a
<i>Azospirillum brasiliense</i>	80.83 b	20.75	147.7 a	2195 a	1380 a	15618 a

* Medium numbers followed by the same letter do not differ statistically between themselves. The Scott-Knott test was applied at 5 % probability.

This result could be associated with the largest volume of roots issued on granular fertilizer. As stated in Gírio et al. (2015) inoculation of growth-promoting bacteria increases the length of the roots, but, do not increase the volume of dry matter.

Nonetheless, the results in which bacterial inoculation and foliar fertilization addressing the density and spore productivity found in granular fertilizer are satisfactory because there is a reduction in its use, resulting in greater cost savings and lower environmental degradation.

The granular nitrogen fertilization promotes benefits more quickly to the soil-plant system, favoring, also, the microorganisms of interest and the increase of organic matter. The highest richness of arbuscular mycorrhizal fungi is directly related to the amount of organic matter in the soil (García, et al., 2014).

About NFB grasses, it is necessary to expand the studies to find out when the bacteria promote a symbiotic relationship with the plant to provide nutrients (JAMES; OLIVARES, 1998).

The application of inoculants formulated by mixing diazotrophic and soil bacteria where they promoted the

increase in productivity of culms and dry matter mass of the sugarcane culture. These results show the economy of nitrogen in the sugarcane culture, since the application of polymers with the mixture of five different bacteria introduced similar measures of productivity increase of culms, in comparison to the control of nitrogen (SILVA et al., 2009).

From the two systems, there were no statistically significant values of minimum productivity (Figure 3), where the planting in stem portions presented productivity of 157 Mg ha⁻¹ while the treatment of planting in PSS presented values of productivity of 119 Mg ha⁻¹.

One of the sources of nitrogen used in commercial compost cover granular fertilizer 10-00-40 and the *Azospirillum brasiliense* inoculant, obtained more expressive results in relation to the foliar fertilizer Biozyme.

The bacteria inoculation of the genus *Azospirillum* in wheat grown in soil with great amount of clay at the National Institute of Agricultural technology in Argentina, it increased the resistance of plants to water stress. Creus et al. (2004).

Gírio et al. (2015) also reached similar results, in the inoculation of sugarcane with growth-promoting bacteria

having physiological effect on the growth of plants. The application of inoculant associated with a low dose of N generated profit and turned out to be profitable in two years studied in sugarcane variety SP80-3280 (PEREIRA, 2011).

In relation to the mycorrhizal colonization, there was no significant difference in the granulated fertilizer,

regardless of the planting system, also in stem portions, there was no difference when evaluating the three nitrogen sources. However, significant differences were found in PSS planting when foliar fertilizations were used and inoculated with *Azospirillum brasiliense* (Table 7).

Table 7. Mycorrhizal colonization rate (%) in sugarcane under two planting systems with different sources of nitrogen.

Planting System	Sources of Nitrogen		
	Granular	Foliar	<i>Azospirillum brasiliense</i>
Pre-sprouted seedlings	19.5 aB*	22.3 aA	22.0 aA
Stem portions	20.5 aA	19.6 bA	19.5 bA

* Medium numbers followed by the same letter do not differ statistically between themselves. The Scott-Knott test was applied at 5 % probability.

The planting of PSS associated with foliar *A. brasiliense* fertilizer presented the best results for colonization that in PSS with granular fertilizer and planting in stem portions with foliar fertilizer, that is for this inoculant because when the plant suffers from lack of nitrogen it seeks association with AMFs present in soil. This fact is proven by de Oliveira et al. (2006) who claims that responses to inoculation are more frequent in the low agricultural aptitude land for the plant they are suffering to severe lack of nutrients. In the studies of (BALDANI et al., 1981), with C3 and C4 grasses, inoculated with *Azospirillum. Brasiliense* and *Azospirillum lipoferum*, there was abundant infection of

the roots. The sugarcane was infected in highest percentage by species *A. brasiliense*. Micropropagated sugarcane seedlings have greater growth and survival when colonized by mycorrhizal fungi (SORIA et al., 2001). Proven by Ortiz et al. (1998), observing the 30 days of transplanting plants colonized by mycorrhizal had 21 % greater height than the ones not colonized. Bacteria of the genre *Azospirillum* has good ability to interact with plants and colonize the entire interior of the roots (MOURA, 2015; STEENHOUDT; VANDERLEYDEN, 2000).

There was statistically significant difference among values of minimum productivity for the varieties studied in the two systems of planting (Table 8).

Table 8. Productivity, Brix and TRS (Total Sugar Recovered) of different varieties of sugarcane under the two planting systems adopted.

Varieties	Productivity (Mg/ha)		Brix (Kg/ha)		TRS (Kg/ha)	
	PSS	Stem portions	PSS	Stem portions	PSS	Stem portions
RB86-7515	129.83 aA	122.83 bA	2216 aA	2017 bA	15483 aA	14204 bA
CTC-4	121.75 aB	181.08 aA	1872 bB	2309 aA	13093 bB	16658 aA
CTC-15	105.66 aB	169.33 aA	2059 aB	2320 aA	17009 aA	16386 aA

The medium-sized, small letters to capital letters and columns to rows. followed by the same letter does not differ statistically between themselves by Scott-Knott test Medium the 5 % probability.

The varieties CTC-4 and CTC-15 presented lower values of productivity when produced under pre-sprouted seedlings system compared to planting system of stem portions and the variety RB86-7515 showed no statistical difference for both of the planting systems.

In relation to productivity in pre-sprouted seedlings, no minimum significant difference was noted between varieties, nonetheless, in the system of stem portions, the RB86-7515 presented lower productivity values compared to the other varieties analysed.

Girio et al., (2015) presents similar results with the variety RB86-7515 where the formation of PSS increases the speed of seedlings sprouting, accumulation of dry materials of the roots and the aerial part plus the productivity, regardless of the amount of the reserve from the buds. And also there was statistically significant difference among brix values, variety x planting system (Table 8).

In relation to levels of brix, the treatments showed similar behaviour to the productivity data, where the CTC-

4 and CTC-15 showed lowest productivities when produced under pre-sprouted seedlings system when compared to planting in stem portions. The variety RB86-7515 showed no statistical difference between the systems.

The variety CTC-4 presented lesser levels of brix than the other varieties when grown under the system of pre-sprouted seedlings. As for the planting system of stem portions, the variety RB86-7515 had the smallest levels of brix, when compared to the varieties TAG-4 and CTC15.

The amount of sucrose present in the broth is the key to a good processing and yield. The reducing sugars, when in high levels, indicate a small advance in ripening of sugarcane (FORTES, 2004).

Marques and Pinto (2013) show that less than 18 % for brix and 85 % for purity, indicate that the culture is immature to harvest.

The same result was found by Rodrigues et al. (2012), evaluating the brix content and fiber in five

sugarcane varieties under planting of stem portions, where the CTC-15 variety obtained a more expressive value.

There was a statistically significant difference in brix values between varieties and nitrogen sources (Table 9).

Table 9. Brix of different varieties of sugarcane under sources of nitrogen.

Varieties	Sources of Nitrogen		
	Granular	Foliar	<i>A. brasiliense</i>
RB86-7515	2050 bA	2118 aA	2181 bA
CTC-4	2108 bA	2102 aA	2063 bA
CTC-15	2340 aA	1889 bB	2340 aA

The medium values. Small letters to columns and capital letters to rows, followed by the same letter does not differ statistically between themselves by Scott-Knott test at 5 % probability.

On the analysis of the interactions between varieties and nitrogen sources, only the variety CTC-15 presented statistically lower values than the other varieties when managed with foliar fertilizer. The other varieties did not presented significant differences, independent of the nitrogen source used.

When assessing the source of nitrogen as an isolated factor, the variety CTC-15 showed the greater productivity when managed with conventional granular fertilizer and with the application of the inoculant *Azospirillum brasiliense*,

however, it was lower than other varieties when handled under foliar fertilization.

Urquiaga et al. (1992), the bacteria-plant interaction and the efficiency of the nitrogen-fixing bacteria are dependent on the genotype of the plant. Schultz et al. (2012), found similar results in experiment that the variety RB86-7515 that was responsive both to nitrogen fertilization and the inoculation of bacterial strains.

There was a statistically significant difference in brix values between variables planting system and nitrogen sources (Table 10).

Table 10. Brix of different planting systems under different sources of nitrogen.

Varieties	Sources of Nitrogen		
	Granular	Foliar	<i>A. brasiliense</i>
PSS	2159 aA	1846 bB	2142 aA
Stem Portions	2173 aA	2226 aA	2247 aA

The medium values. Small letters to columns and capital letters to rows, followed by the same letter does not differ statistically between themselves by Scott-Knott test at 5 % probability.

Analysing the interaction of planting system and the sources of nitrogen, the PSS planting system showed smaller levels of brix when subjected to foliar fertilization. And when comparing the nitrogen sources as an isolated factor, the foliar fertilization presented smaller levels of brix in PSS system than in the stem portions system.

Pereira (2011) shows similar results with the inoculation in the absence of nitrogen fertilization, which promoted significant increase in brix. Arruda et al. (2011), performed joint analysis of variance and linear trend in 141 experiments conducted in the Brazilian Northeast region,

In summary, the study of the levels of TRS, variety CTC-4, presented values inferior than other varieties when grown in pre-sprouted seedlings system.

Observing the systems individually, the variety CTC-4 presented again values inferior than the others when it grown in PSS and the variety RB86-7515 was inferior to the others when grown in stem portions system.

Note that among the varieties cultivated, the RB86-7515 and CTC-15 presented more expressive TRS results. As for the stem portions the varieties CTC-04 and CTC-15 stood out. When comparing the two planting systems there was a significant difference, in which the stem portions showed better results of TRS.

Generally, between planting systems stem portions presented better results when we compare their

noting that the sugarcane plant presented highly significant response to nitrogen fertilization in joint analysis of linear trend of groups of experiments with the same dose of nitrogen.

When evaluating the fiber content of the varieties grown in the experiment, a statistically significant difference was noted between them (Figure 5) where the variety CTC-15 presented larger volumes of fiber than the other varieties analysed.

There was a statistically significant difference in TRS values between the variables varieties x planting system (Table 8).

productivity, brix and TRS. The energy reserve accumulated at the base of the stalk, favors the springs and regrowth of sugarcane. As the productivity of sugarcane, the concentration of reserve energy and the influence of genotypes in harvest season has as an impact in the final concentration of sucrose (SILVA; JERONIMO; LÚCIO, 2008).

Among the nitrogen sources used, the foliar fertilization with biozyme failed to present an expressive result when compared to the commercial fertilizer and with *Azospirillum brasiliense*, due its low nitrogen formulation.

CONCLUSIONS

Although, there was no direct relation between productivity and number of arbuscular mycorrhiza spores, the stem portions planting method showed better results in productivity and in number of spores, especially in the variety CTC-04, it is superior to the others.

REFERENCES

- ARRUDA, L. L. et al. **Comparativo da produtividade e atr entre diferentes variedades de cana de açúcar (*Saccharum officinarum* L.), nas mesmas condições ambientais**. 7ª Mostra científica em Ciências Agrárias. *Anais...* Botucatu, SP: Faculdade de Ciências Agrônômicas, 2011
- BALDANI, J. I. et al. Especificidade na infecção de raízes por *Azospirillum* spp em plantas com via fotossintética C3 e C4. *Pesquisa Agropecuária Brasileira*, v. 16, n. 3, p. 325–330, 1981.
- BERBARA, R. L. L.; SOUZA, F. A.; FONSECA, H. M. A. C. Fungos Micorrízicos Arbusculares: Muito Além da Nutrição. In: FERNANDES, M. S. (Ed.). **Nutrição Mineral de Plantas**. 1ª ed. Rio de Janeiro: Sociedade Brasileira de Ciência do Solo, 2006. p. 432.
- BODDEY, R. M. et al. Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. *Plant and Soil*, v. 252, p. 139–149, 2003.
- CARNEIRO, A. E. V.; TRIVELIN, P. C. O.; VICTORIA, R. L. Utilização da reserva orgânica e de nitrogênio do tolete de plantio (colmo-semente) no desenvolvimento da cana-planta. *Scientia Agricola*, v. 52, n. 2, p. 199–209, ago. 1995.
- COELHO, C. H. M. et al. Identificação de genótipos de cana-de-açúcar quanto ao potencial de contribuição da fixação biológica de nitrogênio. *Agronomia*, v. 37, n. 2, p. 37–40, 2003.
- CONAB. **Acompanhamento da safra brasileira de cana-açúcar 2014/2015**. Brasília, DF.: [s.n.].
- CONSECANA. **Manual de Instruções**. Piracicaba: [s.n.].
- CREUS, C. M.; SUELDO, R. J.; BARASSI, C. A. Water relations and yield in *Azospirillum* inoculated wheat exposed to drought in the field. *Canadian Journal of Botany*, v. 82, n. 2, p. 273–281, fev. 2004.
- DE OLIVEIRA, A. L. M. et al. Yield of micropropagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. *Plant and Soil*, v. 284, n. 1–2, p. 23–32, jun. 2006.
- FILHO, A. C.; NOGUEIRA, M. A. Micorrizas arbusculares em plantas tropicais: café, mandioca e cana-de-açúcar. In: SILVEIRA, A. P. D.; FREITAS, S. S. (Eds.). **Microbiota do solo e qualidade ambiental**. [s.l.: s.n.]. p. 39–56.
- FREITAS, A. W. DE P. et al. Avaliação da divergência nutricional de genótipos de cana-de-açúcar (*Saccharum* spp.). *Revista Brasileira de Zootecnia*, v. 35, n. 1, p. 229–236, fev. 2006.
- GAI, J. et al. Infectivity and community composition of arbuscular mycorrhizal fungi from different soil depths in intensively managed agricultural ecosystems. *Journal of Soils and Sediments*, v. 15, n. 5, p. 1200–1211, 29 maio 2015.
- GARVEY, B. G.; BARRETO, M. J. Changing work and the global commodification of ethanol. *Ateliê Geográfico*, v. 8, n. 1, p. 51–73, 26 mar. 2014.
- GERDEMANN, J. W.; NICOLSON, T. H. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, v. 46, n. 2, p. 235–244, 1963.
- GIOVANNETTI, M.; MOSSE, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, v. 84, n. 3, p. 489–500, mar. 1980.
- GÍRIO, L. A. DA S. et al. Bactérias promotoras de crescimento e adubação nitrogenada no crescimento inicial de cana-de-açúcar proveniente de mudas pré-brotadas. *Pesquisa Agropecuária Brasileira*, v. 50, n. 1, p. 33–43, jan. 2015.
- GOMES, A. A. et al. Relação entre distribuição de nitrogênio e colonização por bactérias diazotróficas em cana-de-açúcar. *Pesquisa Agropecuária Brasileira*, v. 40, n. 11, p. 1105–1113, 2005.
- HUNGRIA, M. **Inoculação com *Azospirillum* brasilense: inovação em rendimento a baixo custo**. Londrina: [s.n.].
- HUNGRIA, M.; NOGUEIRA, M. A.; ARAUJO, R. S. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum* brasilense: An environment-friendly component in the reclamation of degraded pastures in the tropics. *Agriculture, Ecosystems & Environment*, v. 221, p. 125–131, 2016.
- JAMES, E. K.; OLIVARES, F. L. Infection and Colonization of Sugar Cane and Other Gramineous Plants by Endophytic Diazotrophs. *Critical Reviews in Plant Sciences*, v. 17, n. 1, p. 77–119, 1 jan. 1998.
- JASTROW, J. D.; MILLER, R. M.; LUSSENHOP, J. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. *Soil Biology and Biochemistry*, v. 30, n. 7, p. 905–916, 1998.
- KOPPEN, W. **Grundriss der Klimakunde**. Berlin: W. de Gruyter, 1931.

- LANDELL, M. G. DE A. **Sistema de Multiplicação de Cana-de-Açúcar com uso de mudas pré-brotadas (MPB), oriundas de gemas individualizadas.** Campinas: [s.n.].
- MARQUES, T. A.; PINTO, L. E. V. Energia da biomassa de cana-de-açúcar sob influência de hidrogel, cobertura vegetal e profundidade de plantio. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 17, n. 6, p. 680–685, 2013.
- MOHAN, J. E. et al. Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: Mini-review. **Fungal Ecology**, v. 10, n. 1, p. 3–19, 2014.
- MORGADO, I. F. et al. Resíduos agroindustriais prensados como substrato para a produção de mudas de cana-de-açúcar. **Scientia Agrícola**, v. 57, n. 4, p. 709–712, dez. 2000.
- MOURA, J. B. DE. **Diversidade e colonização micorrízica em diferentes usos do solo no Cerrado.** [s.l.] UNB, 2015.
- OLIVER, R. **Interação entre bactérias diazotróficas e doses de n-fertilizante na cultura da cana-de-açúcar.** [s.l.] Universidade Estadual Paulista (UNESP), 2014.
- ORTIZ, R.; FE, C. DE LA; LARA, D. Aportes a la tecnología de micropropagación de la cana de azúcar aplicada en Cuba. III Uso de biofertilizantes y manejo de las vitroplantas en la fase de adaptación. **Cultivos Tropicales**, v. 19, n. 3, p. 49–53, 1998.
- PARANHOS, S. B. **Cana-de-açúcar: cultivo e utilização.** Campinas, SP: [s.n.].
- PEREIRA, W. **Produtividade e qualidade tecnológica da cana-de-açúcar inoculada com bactérias diazotrófica.** [s.l.] UFRRJ, 2011.
- PHILLIPS, J. M.; HAYMAN, D. S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. **Transactions of the British Mycological Society**, v. 55, n. 1, p. 158–161, 1970.
- RAO, I. V. Y. R. Efficiency, Yield Gap and Constraints Analysis in Irrigated vis-a-vis Rainfed Sugarcane in North Coastal Zone of Andhra Pradesh. **Agricultural Economics Research Review**, v. 25, n. 1, p. 167–171, 2012.
- REIS, V. M.; DE PAULA, M. A.; DÖBEREINER, J. Ocorrência de micorrizas arbusculares e da bactéria diazotrófica *Acetobacter diazotrophicus* em cana-de-açúcar. **Pesquisa Agropecuária Brasileira**, v. 34, n. 10, p. 1933–1941, 1999.
- RODRIGUES, R. C. et al. Produtividade e variáveis agroindustriais de cinco variedades de cana-de-açúcar no norte do Espírito Santo. **ENCICLOPÉDIA BIOSFERA**, v. 8, n. 15, p. 1443, 2012.
- SCHULTZ, N. et al. Avaliação agrônômica de variedades de cana-de-açúcar inoculadas com bactérias diazotróficas e adubadas com nitrogênio. **Pesquisa Agropecuária Brasileira**, v. 47, n. 2, p. 261–268, 2012.
- SILVA, F. DE A. S. E.; DUARTE, M. E. M.; CAVALCANTI-MATA, M. E. R. M. Nova metodologia para interpretação de dados de análise sensorial de alimentos. **Engenharia Agrícola**, v. 30, n. 5, p. 967–973, out. 2010.
- SILVA, M. DE A.; JERONIMO, E. M.; LÚCIO, A. D. Perfil hamento e produtividade de cana-de-açúcar com diferentes alturas de corte e épocas de colheita. **Pesquisa Agropecuária Brasileira**, v. 43, n. 8, p. 979–986, 2008.
- SILVA, M. F. DA et al. Inoculantes formulados com polímeros e bactérias endofíticas para a cultura da cana-de-açúcar. **Pesquisa Agropecuária Brasileira**, v. 44, n. 11, p. 1437–1443, nov. 2009.
- SILVEIRA, A. P. D. DA; FREITAS, S. D. S. **Microbiota do Solo e Qualidade Ambiental.** Campinas: IAC, 2007.
- SORIA, A. E. M. et al. Micorrización de plantas micropropagadas de caña de azúcar (*Sacharum officinarum*). **Agricultura Técnica**, v. 61, n. 4, p. 436–443, out. 2001.
- SOUZA, B. R. et al. Arbuscular Mycorrhizal fungi as indicative of soil quality in conservation systems in the region of vale do São Patrício, Goiás. **INTERNATIONAL JOURNAL OF CURRENT RESEARCH**, v. 8, n. 12, p. 43307–43311, dez. 2016.
- STEENHOUDT, O.; VANDERLEYDEN, J. Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. **FEMS microbiology reviews**, v. 24, n. 4, p. 487–506, out. 2000.
- TANEJA, A. P. et al. Sugarcane and its problems. Effect of cropage on the quality of early, mid and late maturing varieties of sugarcane. **Indian Sugar**, v. 36, n. 4, p. 155–159, 1986.
- TRUBER, P. V.; FERNANDES, C. Arbuscular mycorrhizal fungal communities and soil aggregation as affected by cultivation of various crops during the sugarcane fallow period. **Revista Brasileira de Ciência do Solo**, v. 38, n. 2, p. 415–422, 2014.
- URQUIAGA, S.; CRUZ, K. H. S.; BODDEY, R. M. Contribution of Nitrogen Fixation to Sugar Cane: Nitrogen-15 and Nitrogen-Balance Estimates. **Soil Science Society of America Journal**, v. 56, n. 1, p. 105, 1992.
- VIEIRA, P. A. O Brasil nos quadros da economia-mundo capitalista no período 1550-c.1800: Esboço de caracterização através da cadeia mercantil do açúcar Pedro Antonio Vieira 1. **Economia e Sociedade**, v. 3, n. 40, p. 499–527, 2010.

VITTI, A. C. et al. Produtividade da cana-de-açúcar relacionada ao nitrogênio residual da adubação e do sistema radicular. **Pesquisa Agropecuaria Brasileira**, v. 42, n. 2, p. 249–256, 2007.