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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN THE ESTABLISHMENT OF PRE-BROKEN SUGAR CANE

SUMMARY

The objective of this work was to evaluate the influence of arbuscular mycorrhizal fungi on the initial development of pre-sprouted seedlings in three sugarcane varieties (CTC 9004 M, IACSP 955094 and IACSP 962042). The experiment was conducted in a greenhouse in the experimental field and in the agricultural microbiology laboratory of the Goianesia Evangelical Faculty located in the city of Goianésia, Goiás. The experimental design was a 3x2 factorial scheme with 5 replications in which the first factor was constituted by three varieties of sugarcane and the second factor by treatments: sterile soil with inoculation of arbuscular mycorrhizal fungi (AMF) and sterile soil without inoculation. AMF were inoculated directly into the roots of the seedlings. For the determination of growth and development were made count of number of tiller, stem diameter, dry mass of roots, shoot height, mycorrhizal colonization, spore density and identification of associated genera. The data received statistical treatment through the Assistat program. There was statistical difference only in the variety factor, in the variables plant height, spore density and mycorrhizal colonization rate. The genera *Claroideglomus*, *Diversipora* and *Glomus* were identified in all three varieties.

Key words: FMA, *Saccharum spp.*, mycorrhiza.

INTRODUCTION

Brazil is today the largest producer of sugarcane, which makes the country the world leader in raw material production and ethanol production (UNICA, 2017). The Agronomic Institute of Campinas (IAC) launched in 2009 a technique that intends to change the concept of sugarcane plantation in Brazil. The system of pre-budded seedlings (PBS) of cane, as it is known, foresees the rapid production of seedlings, associated with high planting pattern, vigor and uniformity of planting (Landell et al., 2013).

Unlike the conventional system that uses whole stems for planting, the PBS takes to the field only the pre-budded seedlings originated from the cutting

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of the stem, known as wheels, which have an average of 3 cm in length, where the buds are located. The technology has already been adopted by small producers and associations of Goiás and Central region of São Paulo (Souza & Junior, 2013). However, it takes approximately 60 days for the entire process to be completed and the seedlings are perfectly rooted and ready for planting in the field.

The PBS system that uses individualized sugarcane grinding wheels is a rapid multiplication technology that allows the reduction of the volume of vegetal material used in the planting, better control of vigor and high phytosanitary standard, promote sugarcane of excellent clonal standard. The fact of substantially reducing the volume of plant material needed for planting automatically results in a higher gain for the mills, since this material can be used for its production of alcohol or sugar, which will depend on the company's objective (Landell *et al.*, 2013; Xavier *et al.*, 2014).

Unlike traditional planting, seeding pre-sprouted seedlings can be done with adapted machinery which has made the process faster. It is estimated that there is a reduction of 18 to 20 tons (t) of plant material per hectare when the planting is performed with the PBS system, which represents considerable financial gain in the sugar and alcohol industry (Souza & Junior, 2013). Although it is a simple technique, studies on this technique are still lacking, especially those related to the use of plant growth promoting microorganisms, which can be easily applied in encapsulated gems, as predicted in this work. Therefore, the present proposal may contribute significantly to the understanding of the sprouting process and establishment of the seedling in assessing the influence of arbuscular mycorrhizal fungi without its establishment.

According Landell *et al.* (2012) with the advent of mechanical planting, the faults became more frequent and, in order not to result in significant losses in productivity, the volume of seedlings used became very high, being used up to more than 20 t/ha. If a tonne of sugarcane contains 8,000 to 20,000 yolks, it is concluded that the number of yolks per meter is between 24 and 60 yolks, which is therefore an overspending of stalks that could be destined to industry.

Mycorrhizal fungi perform a mutualistic symbiotic association with most cultivated and native plants, benefit the development of the plant due to the greater absorption of water and nutrients, especially phosphorus that has low mobility in the soil (George *et al.*, 1995; Elbon & Whalen, 2014). These microorganisms occupy an important ecological niche in the ecosystems and bring benefits to the associated plant that can increase the rate of seedlings establishments in field conditions (Mohan *et al.*, 2014; Soka & Ritchie, 2015).

When it comes to mycorrhization in sugar cane, it can be noticed the shortage of work, this happens because the culture presents a long and large cycle, which makes it difficult to evaluate it in controlled environments, there are no conclusive results about this mycorrhizal interaction (Reis *et al.*, 1999; Silveira & Freitas, 2007).

This work aimed to evaluate the influence of arbuscular mycorrhizal fungi on the early development of pre-sprouted seedlings in three varieties of sugarcane.

MATERIAL AND METHODS

The experimental design was the 3x2 factorial scheme with five replications, the first factor was constituted by three sugarcane varieties (CTC 9004M, IAC SP 95-5094 and IAC SP 96-2042) and the second factor by treatments: soil sterile with spore inoculation of mycorrhizal fungi and sterile soil without inoculation of spores. The experiment was carried out in the greenhouse and agricultural microbiology laboratory of the Evangelical Faculty of Goianesia.

The sugarcane varieties (*Saccharum officinarum* L.) were chosen according to regional utilization. The buds were from the Jalles Machado mill and were planted in polystyrene trays with commercial Bioplant® substrate and kept in a greenhouse for 30 days.

After 30 days of growth the seedlings were transplanted into 290 cm³ tubes with Bioplant®. Then the seedlings were grown in a greenhouse. After this, they were transferred to 5-liter pots with sterile soil according to the proposed design and with formulated NPK fertilizer (04-30-10). It was conducted inside the greenhouse and irrigation occurred due to the need of the plant.

The spores of arbuscular mycorrhizal fungi (AMF) were extracted from 500 cm³ soil of native cerrado area with characteristics of ciliary forest closed by the wet sieving technique (Gerdemann & Nicolson, 1963), followed by centrifugation in water and 50% sucrose solution. The spores were separated according to their phenotypic characteristics as color, size and shape, composing the different morphotypes under stereoscopic binocular loupe.

For the determination of growth and development the following biometric analyzes were made of plant height, stalk diameter, root dry mass weight, number of tillers, mycorrhizal colonization rate, spore density and identification of associated genera.

In order to determine the percentage of colonization, the roots were clarified and stained with 0.05% Trypan Blue in lactoglycerol (Phillips & Hayman, 1970) and the evaluation of the colonization was made under a stereoscopic microscope, following the technique of intersection of the quadrants (Giovannetti & Mosse, 1980).

For the identification of the AMF genera from the morphological characteristics, the spores were separated according to their morphotypes and assembled on slides with pure polyvinyl lactoglycerol (PVLG) and PVLG mixed with Melzer (1:1 v/v). To support the work of identification, we used original articles of the species description and descriptions of species provided on the website of the "International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi" (Invam, 2018).

RESULTS AND DISCUSSION

The F test shows the significance of the differences of means, in which statistical differences were verified only in the variety factor, in the variables plant height, spore density and mycorrhizal colonization rate (Table 1). No statistical difference was observed between the varieties in relation to root dry weight, stalk diameter and number of tillers. There was no statistical difference regarding inoculation.

Table 1. F test of sugarcane varieties without and with inoculation of arbuscular mycorrhizal fungi

Source of Variation	Root Dough	Height	Diameter	Thorns	Density of Spores	Mycorrhizal Colonization
Variety	0,7811 ns	4.3168 *	1.5444 ns	1.7528 ns	9.5967 **	5.6986 **
Inoculation	0,0117 ns	1.2756 ns	1.2000 ns	0.5506 ns	0.6928 ns	0.4139 ns
Var. x Inoc.	0,5037 ns	0.3289 ns	0.4111 ns	0.7191 ns	1.4135 ns	1.4175 ns

* significant at the 5% probability level ($.01 < p < .05$); ns - not significant ($p \geq .05$)

Tristão *et al.* (2016), when evaluating initial parameters of sugarcane under influence of mycorrhizal fungi, did not observe statistical differences in stem diameter values. In the same study, the authors found a positive influence of the AMF on the variety IAC 91-1099 on the variables of root dry mass and number of tillers.

Andreola *et al.* (1985) evaluated the influence of six species of arbuscular mycorrhizal fungi on the growth and development of three varieties of sugarcane, they found benefits in the development of the varieties, and showed that their efficiency differs according to fungus and variety. Studies have shown that mycorrhizal fungi increase the uptake of nutrients, especially nutrients with low soil mobility, such as phosphorus, copper and zinc, so it is more appropriate to choose varieties that are more efficient in the use of nutrients and which have easier interactions with mycorrhizal fungi arbuscular (Tellechea, 2007). Silva *et al.* (2016) did not verify differences in the diameter of the colon in embaúba seedlings under the inoculation of arbuscular mycorrhizal fungi.

There was difference in relation to plant heights of the three varieties, and the variety IAC SP 96-2042 was statically inferior to the varieties IAC SP 95-5094 and CTC 9004M (Figure 1).

Tristão *et al.* (2016), when evaluating plant height under the influence of mycorrhizal fungi, verified differences among the varieties, and demonstrated that mycorrhizal fungi can develop symbiosis with some varieties more easily than with others. The authors verified higher average values in the varieties IAC SP 95-5094 and IAC 91-1099 and lower average values in IAC SP 97-4039 and IAC SP 95-5000. It is possible to observe that the variety IAC SP 95-5094 has an ease of interaction with arbuscular mycorrhizal fungi. The height of the plant is a

very determinant index for the growth and development in the sugar cane, because it is a factor that will determine the final productivity.

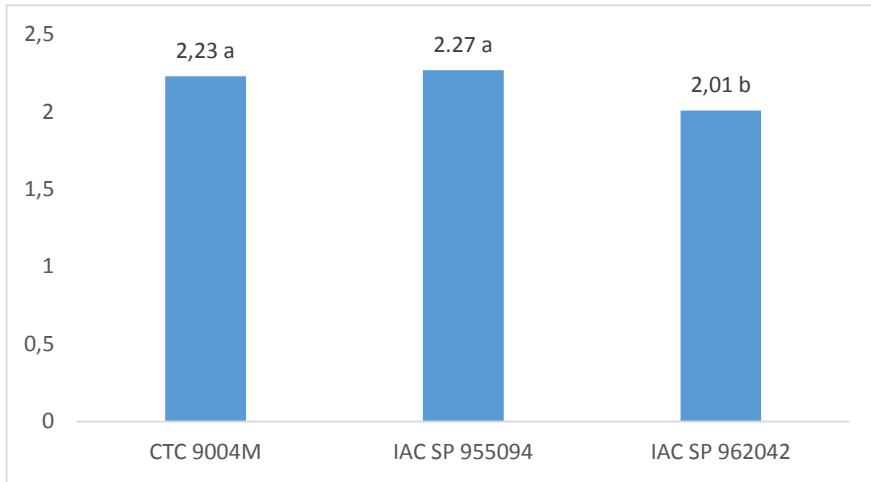


Figure 1. Plant height of three varieties of sugarcane.
CV = 9.67 %

The symbiosis between mycorrhizal seedlings and fungi has positive effects such as increases in CO assimilation rates, transpiration rates and a higher rate of stomatal opening, as well as a higher vegetative growth rate (Schwob et al., 1998; Diniz, 2007; Oliveira et al., 2015).

There was a difference in spore density in samples of sugarcane varieties, being the CTC 9004M variety statically inferior to the varieties IAC SP 95-5094 and IAC SP 96-2042 (Figure 2).

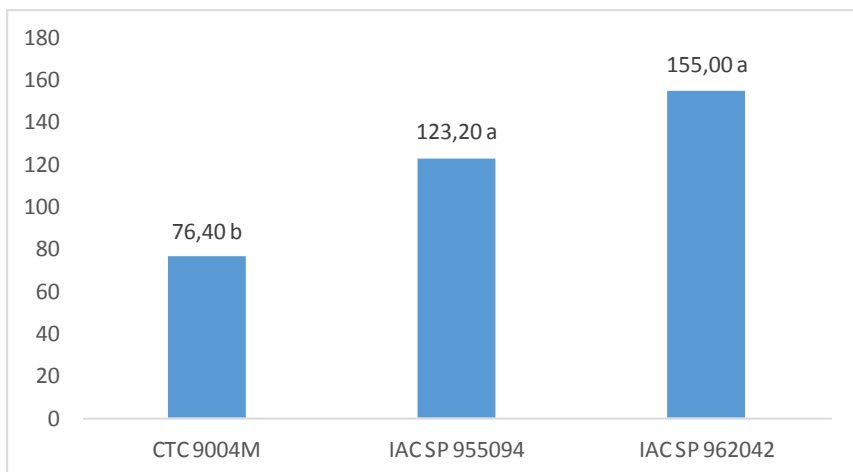


Figure 2. Density of spores in three varieties of sugarcane.
CV = 34.15 %

The papers of Ambrosano *et al.* (2011) and Moura *et al.* (2016) presented that there was no significant difference in spore density in the sugarcane crop.

In relation to the rate of mycorrhizal colonization in samples of sugarcane varieties, a significant difference was observed between the averages, in which the CTC 9004M variety was statically inferior to the varieties IAC SP 95-5094 and IAC SP 96-2042 (Figure 3).

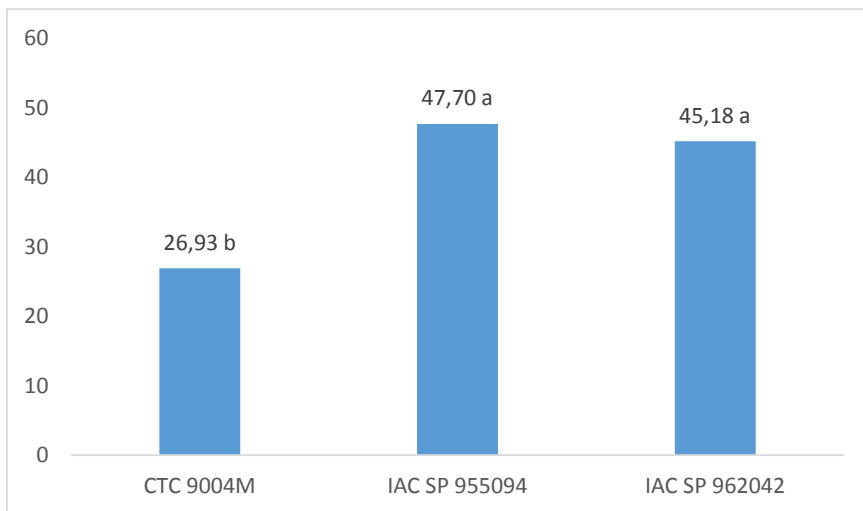


Figure 3. Mycorrhizal colonization rate in three varieties of sugarcane. CV = 31.11 %

Authors report that fungi have an easier mycorrhizal interaction and more sporulation in certain crops and the particular varieties or cultivars, in relation to others. In the work of Silva *et al.* (2016), the number of AMF spores was significant in embaúba seedlings, the same result regarding sporulation was found in the acerola crop when inoculated with mycorrhizal fungi (Balota *et al.*, 2011) and papaya culture (Machineski *et al.*, 2011). Significant values were found in the myriad culture belonging to the family Ericaceae with the cultivars Georgia, Misty and O'neal and lower values in the cultivars of Delite and Climax (Lima, 2014), this reinforces that fungi perform mycorrhizal interaction more easily in some crops and in certain varieties.

In the analyzed samples the genera that interacted with sugarcane varieties were identified, among them the genres: Acaulospora, Claroideglomus, Diversispora, Gigaspora and Glomus (Table 2).

Three of these genera were identified and observed according to Tellechea (2007), the species that are most predominant to interact with sugarcane are of the genres: Acaulospora, Scutelospora, Glomus e Gigaspora. In the work of Miranda (2008) higher rates were identified in the genus Glomus, which is one of the most common genus in the Cerrado.

Table 2. Genus of arbuscular mycorrhizal fungi found associated with the rhizosphere of three sugarcane varieties without and with inoculation of arbuscular mycorrhizal fungi

Genres	Inoculated varieties			Uninoculated varieties		
	CTC	IAC SP	IAC SP	CTC	IAC SP	IAC SP
	9004M	955094	962042	9004M	955094	962042
<i>Acaulospora</i>	-	+	+	-	+	+
<i>Claroideglomus</i>	+	+	+	-	+	+
<i>Diversispora</i>	+	+	+	+	+	+
<i>Glomus</i>	+	+	+	+	+	+
<i>Gigaspora</i>	-	-	+	-	-	-

According Fernandes et al. (2010), the genus *Glomus* was the genus for which a greater number of spores were observed under the pigeon pea, in carrots and beans, *Glomus* and *Gigaspora* were the largest numbers of spores, also.

CONCLUSIONS

The use of arbuscular mycorrhizal fungi in the sprout phase of sugarcane promoted higher results at the height of the varieties in IAC SP 955094 and CTC 9004M in relation to the variety IAC SP 962042.

Mycorrhizal colonization and spore density showed significant values in the varieties IAC SP 955094 and IAC SP 962042.

The genres *Claroideglomus*, *Diversispora* and *Glomus* were identified in all three varieties. The variety IAC SP 962042 presented five genera of mycorrhizal fungi.

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