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Inoculation of arbuscular mycorrhizal fungi on sugarcane varieties in the production of pre-sprouted seedlings

Inoculação de fungos micorrízicos arbusculares em variedades de cana-de-açúcar na produção de mudas pré-brotadas

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ABSTRACT
Brazil is considered the largest producer of sugarcane in the world, the main raw material for the production of ethanol and sugar. The objective was to evaluate the effect of inoculation of arbuscular mycorrhizal fungi in sugarcane varieties in the production of pre-sprouted seedlings. An experiment was carried out in a completely randomized design, in a double factorial 4x2, with four varieties of cane: IACSP95-5000; IAC91-1099; IACSP95-5094 and IACSP97-4039 which interacted with and without the application of arbuscular mycorrhizal fungi on the substrate, with 20 repetitions, totaling 160 experimental units. The use of the mycorrhizal fungi cocktail provided changes in the initial development of the varieties IACSP97-4039, IACSP95-5094 and IACSP95-5000. The inoculation of arbuscular mycorrhizal fungi increased the parameters of the leaf area, the weight of the root dry matter, the adaxial epidermal thickness, the abaxial epidermal thickness, the phloem diameter and the mesophyll thickness.
Keywords: acclimatization; plant morphology; *Saccharum* spp.; soil microbiology.

RESUMO
O Brasil é considerado o maior produtor de cana-de-açúcar do mundo, a principal matéria-prima para a produção de etanol e açúcar. Objetivou-se avaliar o efeito da inoculação de fungos micorrízicos arbusculares em variedades de cana-de-açúcar na produção de mudas pré-brotadas. Um experimento foi conduzido em delineamento experimental inteiramente casualizado, em esquema fatorial duplo 4x2, com quatro variedades de cana – IACSP95-5000, IAC91-1099, IACSP95-5094 e IACSP97-4039 –, as quais interagiram com e sem a aplicação de fungos micorrízicos arbusculares no substrato, com 20 repetições, totalizando 160 unidades experimentais. O uso do coquetel de fungos micorrízicos proporcionou mudanças no desenvolvimento inicial das variedades IACSP97-4039, IACSP95-5094 e IACSP95-5000. A inoculação de fungos micorrízicos arbusculares aumentou os parâmetros da área foliar, o peso da matéria seca da raiz, a espessura epidérmica adaxial, a espessura epidérmica abaxial, o diâmetro do floema e a espessura do mesofilo.
Palavras-chave: aclimatação; microbiologia do solo; morfologia de plantas; *Saccharum* spp.

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INTRODUCTION

Brazil is considered the largest producer of sugarcane (*Saccharum* spp.) in the world (DOSSA, 2009), the main raw material for the sugar and alcohol industry in the production of ethanol and sugar. In the year 2015, according to data from the National Supply Company - CONAB, the average productivity was 73 t ha^{-1}, which represented an increase of 4.3% when compared to the previous year (CONAB, 2019.

The sector has undergone great technological innovations, among them, the System of Pre-Sprouted Seedlings, developed by the Agronomic Institute (IAC), which allowed the reduction in the volume of seedlings used in sugarcane planting. The use of this technology provides greater vigor and uniformity in the planted areas, due to the better use of nutrients and water, leading to less intraspecific competition between plants (LANDELL *et al*., 2012; MATOSO *et al*., 2016).

Currently, it is known that the association between plant roots and certain soil fungi brings great benefits, such as increased vigor and decreased planting time, greater resistance to soil pathogens and greater resistance to abiotic factors (MOREIRA & SIQUEIRA, 2006). Arbuscular mycorrhizal fungi (AMF) belong to the Glomeromycota class and have the ability to colonize most terrestrial vascular plants, forming a symbiotic mutualistic association called mycorrhiza, which favors a greater absorption of water and nutrients, especially the P (FOLLI-PEREIRA *et al*., 2012).

In the sugarcane production system, inoculation with AMFs and the use of suitable substrates allow the production of seedlings with better nutritional and phytosanitary quality (MACHINESKI *et al*., 2009; SOUSA *et al*., 2010), greater tolerance to biotic stresses and abiotic species (MORATELLI *et al*., 2007; SOUSA *et al*., 2010; FOLLI-PEREIRA *et al*., 2012), and, consequently, an increase in sugarcane productivity. Thus, the inoculation of arbuscular mycorrhizal fungi becomes a promising alternative in the production of pre-sprouted seedlings, a fact that has not yet been studied.

In view of the above, this work had the objective of evaluating the effect of the inoculation of arbuscular mycorrhizal fungi on sugarcane varieties in the production of pre-sprouted seedlings.

MATERIAL AND METHODS

The experiment was carried out in August 2015 in a greenhouse at the São Paulo State University (Unesp), College of Agricultural and Technological Sciences, located in the city of Dracena, state of São Paulo, with the geographic coordinates 21°41'57"S and 51°31'58"W and average altitude of 421m. The climate of the region is classified as subtropical Cwa, with mild and dry winter, followed by hot and rainy summer, with average annual temperature of 23.6°C.

The experimental design was completely randomized, in a 4x2 double factorial scheme, with four sugarcane varieties: IACSP95-5000, IAC91-1099, IACSP95-5094 and IACSP97-4039, interacting, with and without the application of arbuscular mycorrhizal fungi on the substrate, with 20 replicates, totaling 160 experimental units. Each replicate was composed of a seedling of viable sugarcane.

The buds came from eight-month-old basic nurseries, free of pathogens, without varietal mixing and produced under the same conditions of cultivation and environment, from the Cana IAC Program in the city of Ribeirão Preto, state of São Paulo. The gems underwent a thermal treatment accompanied by rouging procedures to diagnose diseases. The cuttings of the buds were performed by means of the guillotine system with double disinfected blades, as described

by Xavier *et al*. (2008). In this stage, the best gems were chosen, that is, those that did not present physical damages and had uniformity. The gems were treated with 0.1% Azoxystrobin in solution, immersed for three minutes (LANDELL *et al*., 2012).

The buds were accommodated in sprout boxes containing plant substrate, where they passed the autoclaved sterilization process at 1.0 kgf/cm^2 for 15 minutes. Sprouting boxes were kept for ten days in a greenhouse with an average temperature of 32°C and irrigated with 8.0 mm of water per day.

After sprouting, the acclimation phase 1, with a duration of 21 days, was started, in which the sugarcane gems were individualized, and conditioned in plastic tubes with a volumetric capacity of 250 mL, containing substrate of fertilized vegetable origin with simple superphosphate, potassium chloride and nitrogen fertilizers with slow release, as described by Landell *et al*. (2012). Before planting, a cocktail of soil inocula containing four species of mycorrhizal fungi was applied: *Scutellospora heterograma*, *Rhizophagus clarus* (*Glomus clarum*), *Claroideoglomus etunicatum* (*Glomus etunicatum*), and *Acaulospora morrowiae.* A mass of 100 g of inoculum per kg^{-1} of substrate was applied, as a density of 38 spores per g^{-1} of inoculants, with a total of 3,800 spores.

In the acclimation phase 2, also with a duration of 21 days, the buds were transferred to an external environment with total exposure to the sun, in order to promote their adaptation to the field conditions. At the end of the acclimatization phase, at 42 days after planting the buds, the vegetative parameters were the number of culms, the culms diameter and the leaf area, determined according to the methodology described by Hermann & Câmara (1999).

In order to determine the dry matter of the aerial part and root, all plant material was dried in a forced circulation oven at 70°C, until reaching constant weight (MARAFON, 2012). In addition to the productive analysis, leaves +1 (GALLO *et al*., 1962) fragments were collected from each experimental unit, for the analysis of sugarcane leaf ultrastructure. All fragments of plant tissues received the pertinent procedures for dehydration, diaphanization, inclusion and embedding and, with the help of a microtome, cross sections of 8.0 µm were performed on each tissue fragment.

The slides were observed in an optical microscope with a coupled camera, to perform the measurements of the histological variables, through the analysis program, calibrated with a microscopic ruler at the same magnification as photos and objective lenses, and the following parameters. according to Lisboa *et al*. (2019), were determined: adaxial epidermis thickness; abaxial epidermal thickness; phloem diameter; diameter of the xylem; mesophyll thickness.

The parameters were submitted to analysis of variance by the F test ($p < 0.05$) and their means were compared by the Tukey test at 5% probability. The statistical program Assistat 7.6 Beta (SILVA & AZEVEDO, 2016) was used.

RESULTS AND DISCUSSION

A significant effect was observed in the number of culms, only in the variety factor, with emphasis on IAC91-1099 variety, which presented the highest averages, being higher by approximately 45% in relation to the variety IACSP95-5094, which showed the lowest value for this factor, as shown in Table 1.

Table 1 – Mean values of number of culms; culms diameter and leaf area of sugarcane when inoculated with arbuscular mycorrhizal fungi. Dracena, 2016.

	Number of culms		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	2.30	2.20	2.25 a
IACSP97-4039	1.50	1.70	1.60 b
IACSP95-5094	1.60	1.50	1.55 b
IACSP95-5000	2.00	1.30	1.65 b
MFM(F2)	1.85 A	1.67 A	
CV (%)	36.19		
MSD F1**e F2 ns	0.53111 and 0.28444		
MSD F1xF2 ns	-		
	Culms diameter (cm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	0.53	0.48	0.505 b
IACSP97-4039	0.52	0.48	0.500 b
IACSP95-5094	0.56	0.57	0.565 a
IACSP95-5000	0.52	0.50	0.510 b
MFM(F2)	0.5325 A	0.5075 A	
CV (%)	11.33		
MSD F1**e F2 ns	0.04906 and 0.02627		
MSD F1xF2 ns	-		
	Leaf area (cm²)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	81.46	79.53	80.50 a
IACSP97-4039	76.37	68.60	72.48 a
IACSP95-5094	96.13	68.88	82.50 a
IACSP95-5000	83.91	68.46	76.19 a
MFM(F2)	84.47A	71.37B	
CV (%)	21.74		
MSD F1ns e F2**	14.10 and 7.55		
MSD F1xF2 ns	-		

Lowercase averages followed by the same letter in the column do not differ statistically from each other. Averages followed by the same letter on the line do not differ statistically from one another. ns – Not significant (p>=0.05); *Significant at the 5% probability level (0.01=<p<0.05); **Significant at the 1% probability level (p<0.01). MSD – Minimal significant difference; CV – Coefficient of variation; MFM – mean mycorrhiza factor; MFV – average of the variety factor of sugarcane.

Higher number of sugarcane canes are important factors to guarantee higher productivity, as reported by Silva *et al*. (2008), when studying the tillering and productivity of sugarcane with different cutting heights and harvest times.

For the culms diameter parameter, only the IAC SP95-5094 variety showed the best results, with an increase of approximately 13% in relation to the variety IACSP97-4039. No significant effect of the inoculation of arbuscular mycorrhizal fungi on sugarcane varieties was observed for this parameter. It is worth mentioning that an effect of the inoculation of the arbuscular mycorrhizal fungi on the previously mentioned parameters was expected because, due to a possible increase in nutrient absorption, the number and diameter of culms would be high, as observed by Sousa *et al*. (2010) and Assis *et al*. (2014). The mentioned authors also report that the association of the fungus with the plant increased the resistance of the plant to biotic and abiotic stresses. On the other hand, for Nascimento *et al*. (2016), the association of mycorrhizal fungi improved the bromatological characteristics of the plant.

In the leaf area parameter, a statistical difference was found only for the inoculation factor of the arbuscular mycorrhizal fungi to sugarcane roots, which presented an increase of approximately 18.35% in relation to the non-use of the fungal cocktail. This increase in the leaf area of the plants exposed to the mycorrhizal cocktail may be a result of internal tissue growth, or even a higher efficiency in the functions of these tissues, which activate specific enzymes to transport intra and intercellular nutrients (XIE *et al*., 2016).

As shown in Table 2, in the parameter weight of dry matter of the aerial part, a statistical difference was found only in the variety factor of sugarcane. This result is probably due to the short experimental period because, in only 42 days of evaluation, it was not possible to detect a significant gain of aerial part dry matter.

Table 2 – Mean values of dry matter of the aerial part and root of sugarcane when inoculated to arbuscular mycorrhizal fungi. Dracena, 2016.

	Dry matter of the aerial part (g)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	2.837	3.164	3.0005 ab
IACSP97-4039	3.436	2.993	3.2145 ab
IACSP95-5094	3.608	3.767	3.6875 a
IACSP95-5000	2.937	2.582	2.7595 b
MFM(F2)	3.2045 A	3.1265 A	
CV (%)		28.33	
MSD F1* e F2 ns		0.74666 and 0.39987	
MSD F1xF2 ns		-	
	Matter of the root (g)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	1.359 bA	1.625 aA	1.492bc
IACSP97-4039	1.976 abA	1.606 aA	1.791ab
IACSP95-5094	2.022 aA	2.088 aA	2.055a
IACSP95-5000	1.669 abA	0.948 bB	1.308 c
MFM(F2)	1.7565 A	1.56675A	
CV (%)		31.76	
MSD F1**e F2 ns		0.43938 and 0.23531	
MSD F1xF2*		0.6214 and 0.4706	

Lowercase averages followed by the same letter in the column do not differ statistically from each other. Averages followed by the same letter on the line do not differ statistically from one another. ns – Not significant (p>=0.05); *Significant at the 5% probability level (0.01=<p<0.05); **Significant at the 1% probability level (p<0.01). MSD – Minimal significant difference; CV – Coefficient of variation; MFM – mean mycorrhiza factor; MFV – average of the variety factor of sugarcane.

In the root matter parameter, an interaction between the varieties and the inoculation of the arbuscular mycorrhizal fungi was found, where the variety IACSP95-5094, together with the inoculation of the fungi, provided a gain of approximately 32.82% of root dry matter in IAC91-1099 variety, where the inoculation of arbuscular mycorrhizal fungi did not occur.

The association of arbuscular mycorrhizal fungi did not affect sugarcane in the initial development and in the biochemical components contents, when submitted to associate water stress after 90 days of inoculation (SOUSA *et al*., 2015).

In Table 3, it was observed that, for the thickness of the adaxial epidermis, a significant effect was found only on the variety factor, highlighting the variety IACSP95-5094, that presented a 17.70% thicker epidermis in relation to the variety IACSP95-5000.

Table 3 – Mean values of adaxial epidermis thickness; abaxial epidermal thickness and thickness; phloem diameter; diameter of the xylem; mesophyll thickness of the leaf limbus of the sugarcane when inoculated with arbuscular mycorrhizal fungi. Dracena, 2016.

	Adaxial epidermal thickness (µm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	11.270	12.380	11.828 ab
IACSP97-4039	12.070	10.616	11.345 ab
IACSP95-5094	13.060	11.754	12.410 a
IACSP95-5000	10.490	10.650	10.570 b
MFM(F2)	11.7260 A	11.3500 A	
CV (%)	11.71		
MSD F1* e F2 ns	1.63569 and 0.87105		
MSD F1xF2 ns	-		
	Abaxial epidermal thickness (µm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	9.384	9.480	9.432 ab
IACSP97-4039	11.750	9.648	10.699 a
IACSP95-5094	9.034	7.936	8.485 b
IACSP95-5000	9.734	8.882	9.308 ab
MFM(F2)	9.9755 A	8.9865 B	
CV (%)	15.80		
MSD F1* e F2*	1.81369 and 0.96583		
MSD F1xF2 ns	-		
	Phloem diameter (µm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	5.984	5.332	5.658 a
IACSP97-4039	5.516	4.998	5.257 a
IACSP95-5094	5.664	4.830	5.247 a
IACSP95-5000	5.194	4.130	4.662 a
MFM(F2)	5.5895 A	4.8225 B	
CV (%)	19.19		
MSD F1 ns e F2*	1.20903 and 0.64384		
MSD F1xF2 ns	-		
	Diameter of the xylem (µm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	30.812	30.462	30.637 a
IACSP97-4039	25.012	32.752	28.882 a
IACSP95-5094	29.856	28.860	29.358 a
IACSP95-5000	29.992	32.968	31.480 a
MFM(F2)	28.918 A	31.260 A	
CV (%)	16.09		
MSD F1 ns e F2 ns	-		
MSD F1xF2 ns	-		
	Mesophyll thickness (µm)		
	Inoculated	**Not Inoculated**	**MFV(F1)**
IAC91-1099	620.314	451.226	535.770 a
IACSP97-4039	486.126	525.202	505.664 a
IACSP95-5094	587.286	484.952	536.119 a
IACSP95-5000	639.062	551.698	595.380 a
MFP(F2)	583.1970 A	503.2695 B	
CV (%)	18.82		
MSD F1ns e F2*	128.26810 and 68.30586		
MSD F1xF2 ns	-		

Lowercase averages followed by the same letter in the column do not differ statistically from each other. Averages followed by the same letter on the line do not differ statistically from one another. ns – Not significant (p>=0.05); *Significant at the 5% probability level (0.01=<p<0.05); **Significant at the 1% probability level (p<0.01). MSD – Minimal significant difference; CV – Coefficient of variation; MFM – mean mycorrhiza factor; MFV – average of the variety factor of sugarcane.

For the plants, in general, it is important to present a thinner adaxial epidermis, as it is more translucent for the passage of light, favoring the photosynthetic processes in the leaves (BARROS & SOARES, 2013). On the other hand, the presence of a thicker adaxial epidermis provides greater protection of the plant against stresses caused by herbivory, or even chemical and physical damages (CASTRO *et al.*, 2009).

However, the highlighted variety had a thinner abaxial epidermis. This corresponds to a value of approximately less 26.09% when compared to the variety IACSP97-4039. This result was not expected, considering that the IACSP95-5094 variety had been highlighted among the parameters already mentioned.

The inoculation of the arbuscular mycorrhizal fungi to sugarcane positively influenced the parameter thickness of the abaxial epidermis. This increase reached approximately 11.00%, as shown in Table 3. Due to the anatomical position in the leaf of the plant , this effect of increasing the thickness of the abaxial epidermis may not influence in the same way the passage of light to the mesophyll region of the leaf, when compared to the adaxial epidermis.

The inoculation factor of the arbuscular mycorrhizal fungi provided larger phloem diameter, with an increase of approximately 13.70% for mesophyll thickness in relation to the use of arbuscular mycorrhizal fungi. The function of the phloem is to transport the metabolized sap of the leaves to other regions in the plant and, the possible increase in the concentration of nutrients caused by the inoculation of the fungi to the roots of the sugarcane, may have provided an increase in the phloem diameter (CASTRO *et al.*, 2009).

For the ultrastructural parameters evaluated, it was only for the xylem diameter that no statistical difference between the factors was found. This result was not expected because the mycorrhizal fungi can provide the absorption of a larger volume of solutes of the soils, which would increase the thickness of the xylem vessels. Folli-Pereira *et al*. (2016), studying water stress in sugarcane when subjected to stress caused by nematodes and mycorrhizal fungi, found that the concentration of phosphorus in the plant increased when the soil was more water available, leading to greater transport of solutes from the soil to the aerial part of the plant, corroborating Sousa *et al*. (2010).

It was observed that the greater efficiency in the distribution of sap metabolized via phloem may have provided an increase in the thickness of the leaf mesophyll of pre-sprouted seedlings, which consequently influenced the leaf area of sugarcane, as shown in Table 1. It is worth mentioning that this region presents the highest concentration of photochemical reactions in the plant, as reported by Ramos *et al*. (2014) when studying sugarcane varieties, who found that greater efficiency in the foliar tissues can become an advantage in the productive process of the crop.

The inoculation of arbuscular mycorrhizal fungi occurred satisfactorily in sugarcane varieties IACSP97-4039, IACSP95-5094 and IACSP95-5000.

The inoculation of arbuscular mycorrhizal fungi increased leaf area, root dry matter weight, adaxial epidermis thickness, abaxial epidermal thickness, phloem diameter and mesophyll thickness.

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