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DIAZOTROPHIC ORGANISMS AND ARBUSCULAR MYCORRHIZAL FUNGI IN THE ESTABLISHMENT OF CASSAVA PLANTS

ORGANISMOS DIAZOTRÓFICOS E FUNGOS MICORRÍZICOS ARBUSCULARES NO ESTABELECIMENTO DE PLANTAS DE MANDIOCA

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Abstract

Cassava is an important food base and an excellent alternative source of carbohydrates, especially in developing countries. As it is an easy-to-manage crop, it can be grown in small areas and does not require high soil fertility. This culture has a symbiotic relationship with arbuscular mycorrhizal fungi (AMF), which expand the extent of the roots by the projection of their hyphae, which favors the absorption of water and nutrients from the soil and with nitrogen-fixing bacteria. The objective of this work was to evaluate the interaction of nitrogen-fixing bacteria with arbuscular mycorrhizal fungi at the initial stage of cassava culture. The following parameters were evaluated: the day of sprouting, plant height, root length, plant volume, colonization rate of mycorrhizal fungi and the genera of arbuscular mycorrhizal fungi. Plants inoculated with AMF and diazotrophic bacteria presented the lowest day of sprouting, the highest plant height and longer root length. Mycorrhizal interactions with and without inoculation with nitrogen-fixing bacteria were classified, and mycorrhizal fungi of the genera Acaulospora, Diversispora, Glomus and Gigaspora were classified.

Resumo

A mandioca é um importante alimento na base alimentar, ótima fonte alternativa de carboidratos, especialmente em países em desenvolvimento. Por ser uma cultura de fácil manejo, pode ser cultivada em pequenas áreas e não exige alta fertilidade do solo. Esta cultura possui relação simbiótica com fungos micorrízicos arbusculares (FMA), que ampliam a extensão das raízes pela projeção de suas hifas, o que favorece a absorção de água e nutrientes do solo e com bactérias fixadoras de nitrogênio. O objetivo com este trabalho foi avaliar a interação das bactérias fixadoras de nitrogênio com os fungos micorrízicos arbusculares no estádio inicial da cultura da mandioca. Foram avaliados: o dia da brotação, altura da planta, comprimento das raízes, volume da planta, a taxa de colonização dos fungos micorrízicos e foram classificados os gêneros dos fungos micorrízicos arbusculares. As plantas inoculadas com FMA e bactérias diazotróficas apresentaram o menor dia da brotação, a maior altura da planta e maior comprimento de raízes. Houve interação micorrízica com e sem inoculação com bactérias fixadoras de nitrogênio e foram classificados fungos micorrízicos dos gêneros: Acaulospora, Diversispora, Glomus e Gigaspora.

INTRODUCTION

Cassava *(Manihot esculenta* Crantz), known as macaxeira or aipim, depending on the region, is a species native to Brazil and has its crops spread throughout the country (VALLE, 2005). Brazil occupies the second position in the world production of cassava, accounting for approximately 15% of the production. Considering the entire production chain, cassava cultivation employs approximately two million

people, and family farming accounts for 84% of the country's production. (DEVIDE et al, 2017)

According to IBGE (Brazilian Institute of Geography and Statistics), the area planted with cassava in 2017 was 2.4 million hectares; the northern region is the region with the largest planted area, 860,000 hectares, followed by the northeast region with 800,000 hectares, the southeast region with 148,000 hectares, the southern region with 260,000 hectares and the midwest region with 66,000 hectares (IBGE, 2018).

The cassava yield in Brazil in 2017 was 23,000 tons, and the production per region was as follows: the northern region produced 8,500 tons, the northeast region produced 5,700 tons, the southeast region produced 2,200 tons, the southern region produced 6,000 tons and the midwest region produced 1,200 tons (IBGE, 2018).

Studies conducted with groups of soil microorganisms to evaluate their effects on plant nutrition, particularly with diazotrophic bacteria, have found the occurrence of bacteria belonging to the *genera Azospirillum* and *Klebsiela* in the rhizosphere of cassava culture. (BALOTA et al., 1995)

It was observed that the presence of some species of diazotrophic bacteria increased mycorrhizal colonization from the 30th day and that the mycelial growth of the mycorrhizal fungus was stimulated by exudate released from the cassava plant and exudate of diazotrophic bacteria. (BALOTA et al., 1995)

The objective of this work was to evaluate the initial development of cassava roots inoculated and not inoculated with diazotrophic bacteria of the *genus Azospirillum* and with arbuscular mycorrhizal fungi.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Goianésia, Go, under the coordinates -15° 19' 03" (S) and -49° 07' 03" (W) with an average altitude of 640 meters. The manivas were planted on April 13, 2017, and the work was installed in a shaded environment at 80%. Irrigation was performed whenever necessary by sprinkler to keep plants growing and developing without water deficiency according to recommendations for the crop (LOPES et al., 2010).

The cultivar used was Vassourinha, cataloged as BGM-06 in the Germplasm Bank of Embrapa - CNPMF, Cruz das Almas, BA. Cultivar of upright size, which has as its main characteristic the precocity, is highly used in the region as a table product.

The use of polyethylene bags with a capacity of 1 kg of substrate was adopted. The substrate was sterilized in an autoclave for 90 minutes at a temperature of 120°C to eliminate the presence of microorganisms present in the soil.

The manivas were cut and selected, observing standard size and weight, 12 cm and 40 g.

Extractions of mycorrhizal fungi were performed using the wet extraction method, according to the methodology proposed by Gerdemann & Nicolson (1963) for inoculation of treatments with the presence of fungi, for every 1 kg of sterilized substrate spores extracted from 1 kg of soil were inoculated.

The treatments inoculated with the *bacterium Azospirillun* the manivas were enused in a solution of 50 ml of bacteria with 1 liter of water for 20 minutes, each treatment.

The design adopted in the study was the IHD (fully randomized design), with five replications. There were four treatments. Treatment 01: control; treatment 02: inoculation with diazotrophic bacteria; treatment 03: inoculation with arbuscular mycorrhizal fungi (AMF); treatment 04: inoculation with diazotrophic bacteria + mycorrhizal fungi.

To evaluate the day of sprouting, the experiment was observed daily. At 30 days after planting, the following classes were evaluated: plant

height and root length, in centimeters, with the aid of a tape; plant volume, obtained by the water displacement method, proposed by Sterrett et al. (1968).

For quantification of spores of arbuscular mycorrhizal fungi (AMF), 50 cm^{3} of soil from each treatment was extracted by the wet sieving technique (GERDEMANN & NICOLSON, 1963), followed by centrifugation in water and sucrose solution at 50%. The spores were counted with the aid of binocular magnifying glass and soon after separated according to their phenotypic characteristics, such as color, size and shape, composing the different morphotypes under stereoscopic binocular magnifying glass.

To determine the percentage of colonization, the roots were clarified and coracorated with 0.05% trypan blue in lactoglycerol (PHILLIPS & HAYMAN, 1970), and the evaluation of colonization was made under a stereoscopic microscope, following the technique of intersection of the quadrants (GIOVANNETTI & MOSSE, 1980).

To identify the genera of AMF from morphological characteristics, the spores were separated according to their morphotypes and mounted on blades with pure polyvinyl-lactoglycol (PVLG) and PVLG mixed with Melzer (1:1 v/v). To support the identification work, we used original articles of the description of the species and descriptions of the species provided on the website of the "International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi" (INVAM, 2014). The data were submitted to variance analysis, followed by the Tukey test at 5% probability for comparison of means.

RESULTS AND DISCUSSION

Data on the initial development of cassava plants are presented in Table 1.

Table 01 - (DBAP) day of sprouting after planting. (AP) plant height (cm), (CR) root length (cm), (VP) plant volume (dm^{3}).

TREATMENTS	DBAP	AP	CR	VP
Bacteria + FMA	09 to [1]	40.2 A	31.6 A	0.112 A
Bacterium	13th B	34.8 A	30.2 AB	0.092 A
FMA	14BC	27.6 B	28.8 B	0.064 B
Control	16th C	24.4 B	28 B	0.066 B
DMS	3,18	5,95	2,6	0,24

[1]: Followed averages of the same letter in the column do not differ from each other, according to Tukey's 5% probability test.

Inoculation with *Azospirillum* significantly influenced the initial establishment of cassava. The treatment inoculated with diazotrophic bacteria and AMF showed the shortest time for sprouting, on average 09 days after planting. The treatment that was inoculated with the bacteria and the treatment that was inoculated with AMF did not differ statistically; they presented 13 and 14 days for sprouting after planting, respectively. The treatment with AMF did not differ statistically from the control treatment, which was sprouted at 16 days after planting. (Table 01).

At plant height, it was observed that there was no significant difference between the treatment where diazotrophic bacteria and AMF were inoculated and the treatment where only diazotrophic bacteria were inoculated, presenting 40.2 and 34.8 cm, respectively, but differed statistically from the treatment inoculated with AMF 28.8 cm and the control treatment 28 cm.

In the length of the roots, the treatment inoculated with bacteria and AMF presented an average of 31.6 cm, which did not differ statistically from the treatment inoculated with bacteria (30.2 cm). This did not differ statistically from the other treatments, which had averages of 28.8 cm for the AMF treatment and 28 cm for the control treatment.

Balota et al. (1999) observed that under natural conditions, cassava culture has been associated with a large number of species of fungi micorrizos, but only a few species are efficient. Other studies corroborate these results; in crops in the southern region of Minas Gerais, 181 spores per 100 g of soil were observed, with a predominance of *A. scrobiculata* and *Gigaspora sp.* (SIQUEIRA et al., 1989).

In the volume of the plant, the treatment with bacteria and AMF presented 0.112 dm^3, the treatment with bacteria presented 0.092 dm^3, which did not differ statistically from each other but differed from the other treatments. The treatment with AMF presented 0.064 dm^3, and the control treatment presented 0.066 dm^3. Several authors (BALOTA et al, 1995; BALOTA et al, 1999; SIQUEIRA et al, 1989) found results in which Mycorrhizal fungi are associated with cassava. Balota et al. (1995) found an association of diazotrophic bacteria in the cassava rhizosphere.

The amount of arbuscular mycorrhizal fungi spores in the rhizosphere and the root colonization rate were not affected by the inoculation of diazotrophic bacteria, as shown in Table 02.

Table 02. Density and colonization rate of arbuscular mycorrhizal fungi in cassava culture.

Treatments	Dens. spores (50 cm^3)	Root colonization rate (%)
Control	0 b	0 b
Diazotrophic bacteria	0 b	0 b
Mycorrhizal fungi	123.2 to	47.7 to
Bacteria + Fungi	155 a	45.19 to

Note: Media followed by distinct letters between lines differed in the Tukey statistical test at 5% probability

Authors report that fungi have more ease of mycorrhizal interaction and more sporulation in certain cultures and certain 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, and the same result in relation to sporulation was found in acerola cultures when inoculated with mycorrhizal fungi (BALOTA et al., 2011) and maurose cultures (MACHINESKI et al., 2011).

Symbiosis between mycorrhizal seedlings and fungi has positive effects, such as increases in CO assimilation rates, transpiration rates and stomatal conductance, in addition to greater vegetative growth (SCHWOB et al., 1998; DINIZ, 2007; OLIVEIRA et al., 2015)

Andreola et al. (1985) evaluated the influence of six species of arbuscular mycorrhizal fungi on the growth and development of three varieties of sugarcane and verified benefits in the development of varieties, and their efficiency varied according to the fungus and variety. Studies show that mycorrhizal fungi increase the absorption of nutrients, especially nonmobile nutrients that are in the soil, such as phosphorous, copper and zinc, and it is most appropriate to choose more efficient varieties in the use of nutrients that present easier interactions with arbuscular mycorrhizal fungi (TELLECHEA, 2007).

Table 03. Genera of arbuscular mycorrhizal fungi found associated with cassava rhizosphere without and with the inoculation of diazotrophic bacteria.

	Treatments			
Gender	Control	Diazotrofic bacteria	Mycorzicos Fungi	Bacteria + Fungi
Acaulospora	.	.	.	**x**
Claroideglomus	.	.	.	.
Diversispora	.	.	**x**	**x**
Glomus	.	.	**x**	**x**
Gigaspora	.	.	**x**	.

It was observed that the absence of inoculation of diazotrophic bacteria benefited the emergence of the genus *Gisgaspora,* and with the presence of bacteria, the genus *Acaulospora* benefited. In the treatment that was inoculated with mycorrhizal fungi and inoculated with bacteria + mycorrhizal fungi, the presence of *the genus Glomus* is *Diversispora was found.* The treatments without inoculation (control) and inoculated only with diazotrophic bacteria did not have the presence of fungi since the soil had been sterilized and not inoculated later. (table 03).

Balota et al. 1997, when working with diazotrophic bacteria and arbuscular mycorrhizal fungi in cassava culture, concluded that the presence of bacteria has a stumulatory effect only for some species of AMF.

According to Fernandes et al. (2010), the genus Glomus was the genus for which, but a greater number of spores under the guandu, in carrot and beans, Glomus and Gigaspora were the highest numbers of spores, equally. There are few studies demonstrating the symbiosis between sugarcane seedlings and mycorrhizal fungi, which is an important field to explore.

CONCLUSIONS

Inoculation of cassava with diazotrophic bacteria in the presence of mycorrhizal fungi influenced the day of sprouting.

Plant height was higher when inoculated with diazotrophic and AMF bacteria.

The roots developed more in the presence of diazotrophic bacteria.

There was a gain in the volume of plants inoculated with diazotrophic bacteria and AMF.

The presence of diazotrophic bacteria did not influence the amount of mycorrhizal fungi present in the rhizosphere. The presence of bacteria influencing the presence of fungi of the genus *Acaulospora* delayed the presence of the genus *Gigaspora.*

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