Agronomic Journal
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ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH PEPPER TEN LINES RHIZOSPHERE CHILLIES *Capsicum frutescens*

FUNGOS MICORRÍZICOS ARBUSCULARES ASSOCIADOS A RIZOSFERA DE DEZ LINHAGENS DE PIMENTA MALAGUETA *Capsicum frutescens*

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Info

Recebido: 01/2019
Publicado: 06/2019
ISSN: 2595-6906

Palavras-Chave
FMAs, pimenta, identificação
Keywords:
MAF, Pepper, identification.

Resumo

O negócio de pimentas é um importante segmento do mercado agrícola brasileiro, com forte expressão na indústria alimentícia, farmacêutica e cosmética. Objetivou-se com este trabalho determinar a taxa de colonização micorrizica, densidade de esporos e identificação de gêneros de fungos micorrízicos associados à rizosfera de dez linhagens de pimentas malagueta (Capsicum frutescens). O delineamento experimental foi em inteiramente casualizado, com 10 tratamentos e 4 repetições, sendo as dez linhagens de Capsicum frutescens:IFET-1121; IFET-1109; IFET-1129; IFET-1119; IFET-1117; IFET-1137; IFET- 1131; IFET-1127; IFET- 1125 e IFET-1111. Os esporos de fungos micorrízicos arbusculares (FMAs) foram extraídos do solo utilizando-se 50 cm^3 de cada amostra composta, pela técnica de peneiramento úmido. A determinação da porcentagem de colonização micorrízica deu-se através da técnica de interseção dos quadrantes. A identificação das espécies de fungos micorrízicos foi por comparação morfológica com base nas descrições das culturas de referência presentes no International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. Os dados de número de esporos e colonização micorrízica foram submetidos à análise estatística clássica por meio do programa Assistat (2016). Foram identificados os gêneros Acaulospora, Claroideoglomus, Diversispora, Scutellospora, Sclerocystis, Glomus, Funneliformis e Gigaspora associados à rizosfera das linhagens de Capsicumfrutescens. Os gêneros Glomus, Acaulospora e Claroideoglomus foram encontrados em todas as linhagens analisadas. A linhagem IFET – 1127 apresentou maiores valores de densidade de esporos quando comparado ás demais linhagens estudadas Não foi identificado diferença mínima significativa nos valores de taxa de colonização micorrízica entre as linhagens investigadas.

Abstract

The peppers business is an important segment of the Brazilian agricultural market, with strong expression in the food, pharmaceutical and cosmetic industries. The objective of this work was to determine the mycorrhizal colonization rate, spore density and mycorrhizal fungi genotypes associated with the rhizosphere of ten lines of chilli peppers (Capsicum frutescens). The experimental design was completely randomized, with 10 treatments and 4 replicates, with the ten strains of Capsicum frutescens: IFET-1121; IFET-1109; IFET-1129; IFET-1119; IFET-1117; IFET-1137; IFET-1131; IFET-1127; IFET-1125 and IFET-1111. The spores of arbuscular mycorrhizal fungi (AMF) will be extracted from the soil using 50 cm^3 of each composite sample, using the wet sieving technique. The determination of the percentage of mycorrhizal colonization occurred through the technique of intersection of the quadrants. The identification of mycorrhizal fungi species was by morphological comparison based on the descriptions of the reference cultures present in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi. The spore number and mycorrhizal colonization data will be submitted to classical statistical analysis using the Assistat program (2016). The genus Acaulospora, Claroideoglomus, Diversispora, Scutellospora, Sclerocystis, Glomus, Funneliformis and Gigaspora associated with the rhizosphere of the Capsicum frutescens strains were identified. The genera Glomus, acaulosporand Claroideoglomuswere found in all strains analyzed. The IFET - 1127 strain presented higher spore density values when compared to the other strains studied. No significant difference was found in the values of mycorrhizal colonization rate among the investigated strains.

INTRODUCTION

The species of chilies and peppers of the genus Capsicum originate in the Americas and were already consumed more than 7,000 years ago in Mexico. According to Carvalho et al. (2006), it was observed that some indigenous tribes used ground pepper mixed with ash as an efficient method of preserving seeds of other traditionally cultivated species.

The peppers business is an important segment of the Brazilian agricultural market, as a strong expression in the food, pharmaceutical and cosmetic industries. In the state of Goiás, pepper occupies a prominent position, with Ceasa de Goiânia being the only supply center in the country to discriminate all types of peppers and to make quotations separately (RIBEIRO, 2008).

It is believed that there are about 30 species in the genus Capsicum, but only five are domesticated and four of these occur in Brazil, the other species are semi-domesticated or wild, in relation to color, fruit type, size, , 2004). Most of these species are part of the genetic heritage of Brazilian biodiversity.

The genetic improvement of plants, in general, aims to identify and select superior genotypes when in production, aiming to obtain what is called plant ideotype (BUENO et al, 2006).

The public programs of genetic improvement Capsicum in Brazil are concentrated in the vegetable crops and have been successful in obtaining genetic material that meet the demands of consumers and producers. However, genotypes specifically adapted to the conditions of the central and northern regions of Goiás have not yet been developed (Vieira et al., 2015).

Even though most of the production is commercialized in regional and local markets, and still with their importance, data on production and commercialization of peppers in Brazil are scarce, but it does not reflect the economic reality of these vegetables (Domenico et al., 2010).

The research in the field of microbiology in peppers is still incipient, little is known of the dynamics of mycorrhizal fungi with different commercial varieties. Mycorrhizae are formed through the association of the host plant and fungus (MERRYWEATHER; FITTER, 1998). Through this symbiosis, mycorrhizae are capable of increasing plant nutrition, increasing the area of root exploration and bringing benefits to the productivity of the associated plants (OLIVEIRA, MOURA, SOUZA, & FURQUIM, 2017, SOUZA & SILVA, 1996).

The understanding of the environmental relationships between pepper cultivars and the endemic mycorrhizal fungi of the cerrado is important for elucidating these issues and for the future development of management techniques that can reduce costs, environmental impacts and maximize productivity gains.

The objective of this work was to determine the rate of mycorrhizal colonization, spore density and identification of genus of mycorrhizal fungi associated with the rhizosphere of ten lines of chilli peppers (Capsicum frutescens).

MATERIAL AND METHODS

The experiment was installed in the experimental field of the Goiano Federal Institute - Câmpus Ceres, located at Rodovia GO 154, Km 3 Rural Area Ceres-GO.

The analyzes of spore density, mycorrhizal colonization rate and identification of the genera of associated mycorrhizal fungi were performed at the Agricultural Microbiology Laboratory of Goianésia 's Evangelical Faculty, located at Avenida Brasil, n1000, Bairro Covoá, Goianésia - GO.

The experimental design was completely randomized, with 10 treatments and 4 replicates, with the ten Capsicum frutescens strains: IFET-1121; IFET-1109; IFET-1129; IFET-1119; IFET-1117; IFET-1137; IFET-1131; IFET-1127; IFET-1125 and IFET-1111. The lines come from the genetic improvement program of the Goiano IF - Campus Ceres. The access IFET 127 that gave origin to the lineages belongs to the species Capsicumfrutescens, has elongated fruits, with smooth bark and pendant and orange color, picanciamédia and green leaves. The lineages belong to the peppers of the Malagueta group.

The experiment was grown under greenhouse conditions. Seeding was carried out in styrofoam trays of 128 cells containing commercial PlantmaxR substrate and one seed per cell. When the seedlings reached between 10 and 15 cm in height and 4 to 6 final leaves were transplanted to vessels of 10.0 L capacity with a substrate mixture with the proportion of 75% fine sifted earth, 25% bovine manure tannate, 5 g of Basacote® (N-13%, P2O5-6%, Cu-0.05%, Fe-0.26%, Mg-1.2%, Mn - 0.06%, Mo-0.015%; 10%), 10 kg of dolomitic limestone, 12 g of potassium chloride (60% K2O) and 30 g of magnesium thermophosphate (yoorin) as a source of phosphorus (18% P2O5). Irrigation was performed by micro sprinkler with four irrigations daily for three minutes each. As a cultural management, training pruning was adopted, where all the branches and shoots were cut below the bifurcation of the plant, always being careful to sterilize the pruning equipment with sanitary water and 70% alcohol. FMA species were not inoculated.

For the analysis of mycorrhizal colonization, spore density and identification of AMF genera, a portion of rhizosphere soil and roots of the plants analyzed were collected.

The spores of arbuscular mycorrhizal fungi (AMF) were extracted from the soil using 50 cm^3 of each composite sample, using 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 loupes.

In order to determine the percentage of mycorrhizal colonization, the roots were clarified and stained with 0.05% Trypan blue in lactoglycerol (PHILLIPS & HAYMAN, 1970) and the colonization evaluation was done under a stereoscopic microscope, following the technique of intersection of the quadrants (GIOVANNETTI & MOSSE, 1980).

For identification of the genera of AMF from the morphological characteristics, spores were separated according to their morphotypes and mounted on slides with pure polyvinyl lactoglycerol (PVLG) and PVLG mixed with Melzer (1: 1 v / v). The identification of the genus of mycorrhizal fungi was carried out following the descriptions of the reference cultures present in the International Culture Collection of Arbuscular and

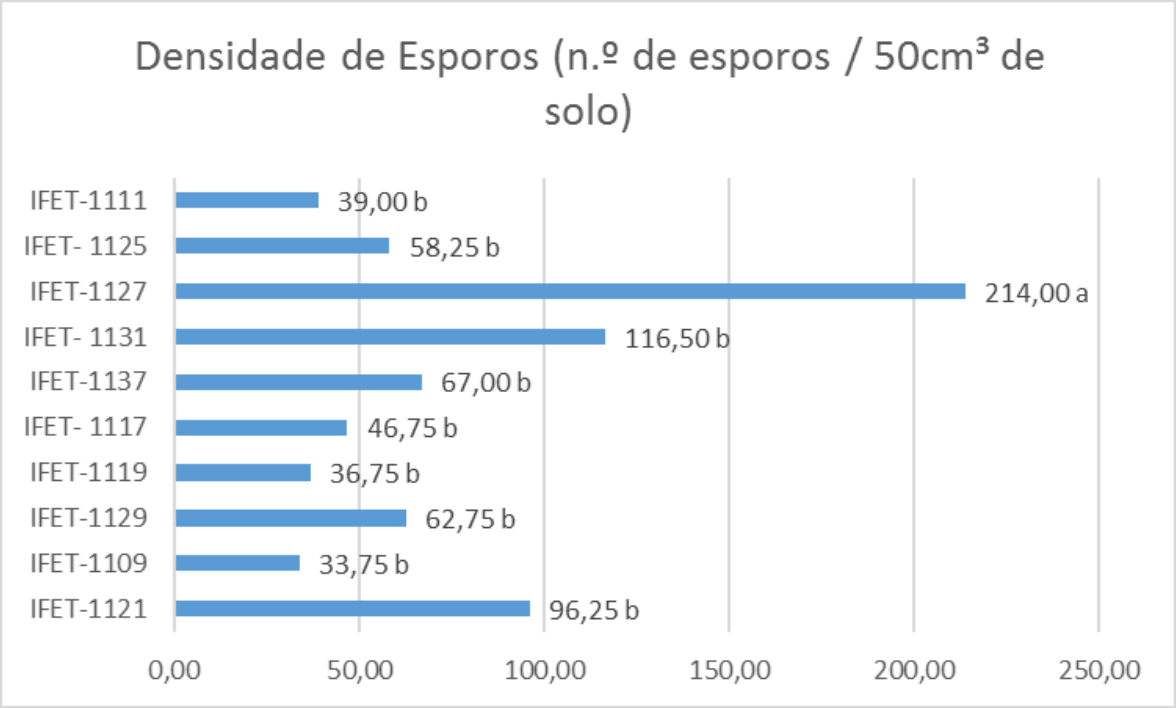
Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, 2014).

The spore number and mycorrhizal colonization data were submitted to classical statistical analysis using the Assistat program (2016).

RESULTS AND DISCUSSION

When investigating the spore density of arbuscular mycorrhizal fungi, it was possible to observe a significant statistical difference in C. frutescens strains. With the exception of the IFET - 1127 strain that presented higher spore density values than the other strains (Figure 1).

Figure 1. Density of spores in different strains of Capsicum frutescens.



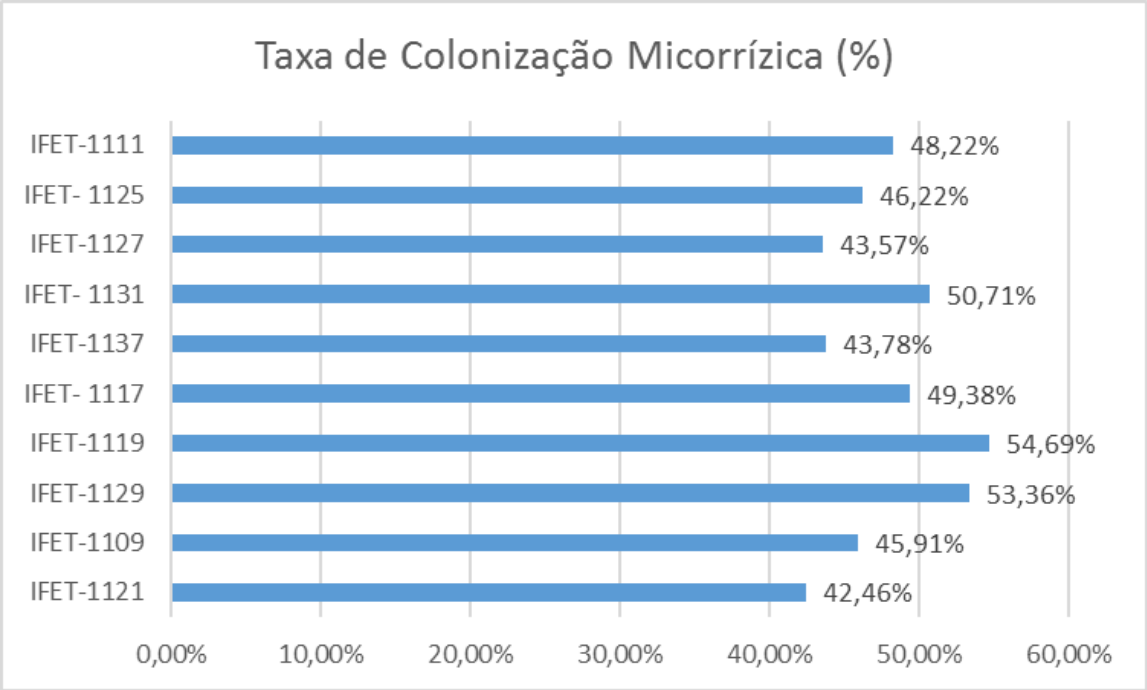
The presence of arbuscular mycorrhizal fungi in pepper rhizosphere promotes the growth and vegetative development of these species. Naturally this plant species has high affinity with native species of mycorrhizal fungi (SANCHEZ & ROQUE, 2016). The same authors, when investigating the influence of fungi on pepper yield, verified positive influence of fungi on biomass gain and yield, reporting the absence of fruits in treatments without the presence of mycorrhizal fungi.

Ortas et al. (2011) and CARDONA ET AL., (2008) found no statistical difference between different pepper varieties, demonstrating that this plant genus has low symbiotic specificity with mycorrhizal fungi.

Although the amount of research on mycorrhizal fungi is large, there is no clarification about the interaction of FMA with different strains of the same plant species (AQUINO, 2003).

No significant difference was found in the values of mycorrhizal colonization rate among the lines investigated (Figure 2).

Figure 2. Rate of Mycorrhizal colonization in different strains of Capsicum frutescens.



Sánchez and Roque (2016) found no differences between the means of mycorrhizal colonization when evaluating different Capsicum cultivars.

Ortas et al. (2011) verified a higher colonization in plants that were inoculated in the platio than in the cover, demonstrating that the proximity with spores in the soil has a positive influence on the amount of Glomus mycorrhizal fungi in C. anum, of colonized roots.

Nutritional aspects may also interfere with the rate of colonization. Elevated P levels in soil impair root colonization by the fungus (Maiti, Toppo, and Variar 2011). Beltrano et al. (2013) evaluated the influence of phosphorus levels on the mycorrhizal colonization rate in C. anum and verified that the increase of phosphorus in fertilization impairs colonization.

Cardona (2008) found a mean of 83% mycorrhizal colonization in Capsicum in soils of the Colombian Amazon, however, also did not verify difference in colonization values among Capsicum species. This confirms that there are not enough physiological differences between C. frutescens strains to have large differences in mycorrhizal colonization values.

However, water stress is an environmental factor that promotes mycorrhizal colonization (Morte, Lovisolo, and Schubert 2000). Davies et al. (2002) found results that corroborate this assertion when assessing the influence of water stress under the colonization rate in C. anum.

Such factors may be the reason why the colonization rate did not present significant difference. The experimental conditions did not put the vegetables under stressful conditions, thus not having to establish the symbiosis with the fungus.

Eight genera of arbuscular mycorrhizal fungi were found in the rhizosphere of the ten lines investigated (Table 1).

Table 1. Cultures of arbuscular mycorrhizal fungi found in rhizosphere of ten lines of chilli pepper (Capsicum frutescens).

Gender	IFET-1121	IFET-1109	IFET-1129	IFET-1119	IFET-1117	IFET-1137	IFET-1131	IFET-1127	IFET-1125	IFET-1111
Acaulospora	+	+	+	+	+	+	+	+	+	+
Claroideoglomus	+	+	+	+	+	+	+	+	+	+
Diversispora	-	+	+	-	+	+	+	+	+	+
Scutellospora	+	+	+	-	-	+	-	+	-	-
Sclerocystis	+	+	-	+	+	-	+	-	+	+
Glomus	+	+	+	+	+	+	+	+	+	+
Funneliformis	-	-	-	-	-	-	+	-	-	-
Gigaspora	+	+	+	+	+	+	+	+	-	+
Total de Gêneros	6	7	6	5	6	6	7	6	5	6

The genera Glomus, Acaulospora and Claroideoglomus were found in all strains analyzed. When evaluating species of mycorrhizal fungi in different species of pepper, Sánchez and Roque. (2016) also found these genera associated with Chilli pepper rhizosphere.

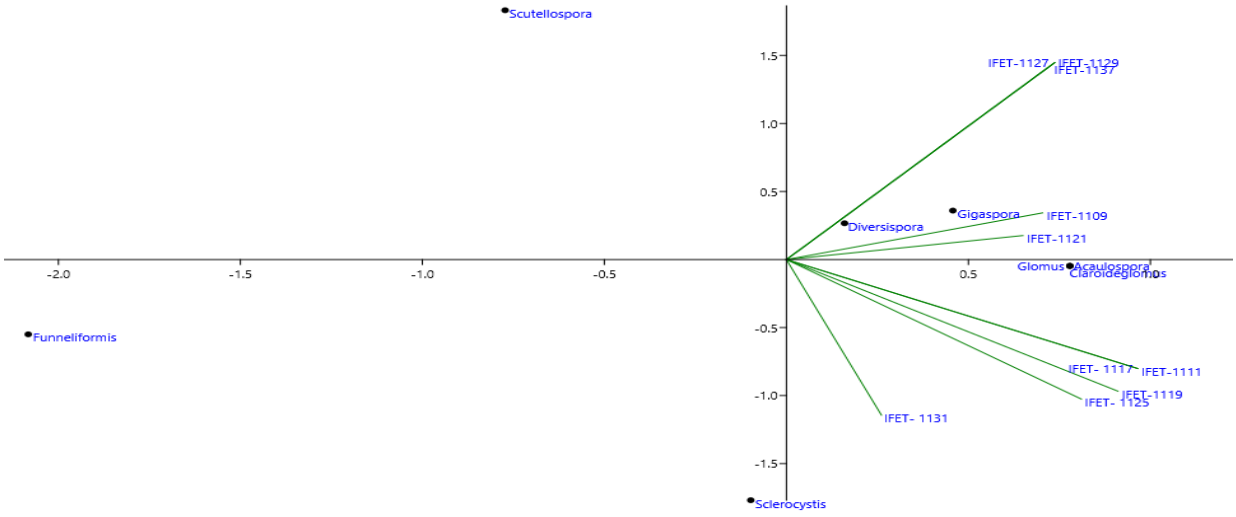
Cardona., (2008) evaluated the occurrence of different species of mycorrhizal fungi in C. frutescens also found the Glomus and Acaulospora genera present in the rhizosphere of the investigated plants.

However, the genus Funeliformis was found only in the line IFET 1131. This behavior indicates a coincidence in the association of this genus of fungus with C. frutescens.

Due to the fact that they differ from other strains, their presence may have been due to contamination, which occurred during the rooting period of the cuttings, through wind or irrigation water (WEBER & AMORIM, 1994).

Figure 3 shows the analysis of principal components between the investigated lines and the genera of FMA found in rhizosphere.

Figure 3. Analysis of major components of the genera of arbuscular mycorrhizal fungi and different strains of Capsicum frutescens.



The genera Funneliformis, Sclerocustis and Scutellospora distance themselves from the studied strains. This indicates that these genera do not form mycorrhizal association with the pepper strains and were found casually in the samples. The other genera have similar proximity with all studied strains.

The most commonly associated genera are glomus, Gigaspora, and Acaulospora, as well as the genus Glomus, as well as verified in the results.

CONCLUSIONS

The genus Acaulospora, Claroideoglomus, Diversispora, Scutellospora, Sclerocystis, Glomus, Funneliformis and Gigaspora associated with the rhizosphere of the Capsicum frutescens strains were identified.

The genera Glomus, Acaulospora and Claroideoglomus were found in all strains analyzed.

The IFET - 1127 strain had higher spore density values when compared to the other strains studied.

No significant difference in the values of mycorrhizal colonization rate was identified among the investigated strains.

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