

Morphological pattern of colonization by mycorrhizal fungi and the microbial activity observed in Barbados cherry crops

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ABSTRACT: *Our objective was to evaluate the morphological pattern of colonization by arbuscular mycorrhizal fungi (AMF) as well as to study the microbiological activity of the soil on family farms where Barbados cherry was cultivated. Soil and root samples were selected from four areas in the municipality of Maranguape-CE where Barbados cherry was grown, which were named according to the age of plants in the following manner: A1-3, A2-3, and A3-3 (3 years), and A4-2 (2 years). After sampling, the arbuscular mycorrhizal colonization, morphological colonization pattern, basal soil respiration (SBR), and density of mycorrhizal spores (DS) were analyzed. The Paris-type morphological pattern was predominant in the root system of Barbados cherry; the affinity of this pattern in the culture was clear. Time of installation and management of Barbados cherry orchards in family farming areas promoted reduction in SBR. P levels in the soil may have negatively influenced root colonization and density of mycorrhizal spores.*

Key words: *Malpighia emarginata D.C., family farming, mycorrhizal.*

Padrão morfológico de colonização por fungos micorrízicos arbusculares e atividade microbiana em cultivos de acerola

RESUMO: *Objetivou-se avaliar o padrão morfológico de colonização por Fungos Micorrízicos Arbusculares (FMA), bem como a atividade microbiológica do solo sob cultivo da aceroleira em áreas de agricultores familiares. Para as coletas de solos e raízes foram selecionadas quatro áreas com aceroleira no município de Maranguape-CE, essas foram denominadas de acordo com a idade das plantas A1-3, A2-3 e A3-3 (3 anos) e A4-2 (2 anos). Após as coletas foi analisado a colonização micorrízica arbuscular, o padrão morfológico de colonização, a respiração basal do solo (RBS) e a densidade de esporos micorrízicos (DE). O padrão morfológico do tipo Paris foi predominante no sistema radicular da aceroleira, ficando claro a afinidade desse padrão na cultura. O tempo de instalação e o manejo do pomar da aceroleira nas áreas de agricultura familiar promoveu uma redução na RBS. Os teores de P no solo podem ter influenciado negativamente a colonização radicular e a densidade de esporos de fungos micorrízicos arbusculares.*

Palavras-chave: *Malpighia emarginata D.C., agricultura familiar, micorriza.*

INTRODUCTION

Acerola numbers have increased in recent years in Brazil, and the species is grown in virtually all regions of the country. Its fruits are mainly sold fresh and in the form of frozen pulp. The acerola tree is considered as a drought-resistant and rustic plant. Owing to these characteristics, this plant species adapts to conditions of young and low-fertility soils, such as Brazilian Northeast soils (FIGUEIREDO NETO et al., 2014).

The increased demand for fresh acerola and its pulp in the Brazilian Northeast has led

to an increase in the number of biotechnological research studies on sustainable soil management, microbiological activity, and the association between acerola trees and arbuscular mycorrhizal fungi (AMF). Soil microorganisms are crucial for soil organic matter cycling (ROSA et al., 2017) and nutrient absorption, and their presence leads to the enhancement of nutrient uptake, microbiological activity, and an increase in acerola production.

These studies aim to develop biotechnological methods for the use of soil microorganisms in both sustainable soil

management and acerola crop production associated with AMF because this plant is mycotrophic, that is, it requires mycorrhizal association to enhance nutrient absorption, particularly of phosphorus, thereby increasing the plant growth and productivity (BALOTA et al., 2011; BAUM et al., 2015). However, limited number of studies on acerola growth and symbiosis with AMF have been published in the literature thus far, despite its importance. Another relevant fact is the absence of studies regarding the mycorrhizal morphological patterns prevalent in acerola roots, which underlines the importance of the present pioneering study on acerola growth.

This symbiotic association also increases the tolerance of acerola plants to biotic and abiotic environmental stresses during the nursery-to-field transplantation phase. Thus, mycorrhization of acerola seedlings enhances their initial growth in the nursery, allowing increased plant growth and nutrition in the field (FERREIRA et al., 2015). Benefits of acerola-AMF symbiosis are related to the morphological formation of intra- and intercellular endophytic structures. Morphology of these structures in arbuscular mycorrhizae has been classified as “*Arum*”, “*Paris*”, and “intermediary”. The “*Paris*” type has intracellular hyphae, which can be folded; “*Arum*” is observed in intercellular hyphae and arbuscules; and “intermediary” has both “*Arum*” and “*Paris*” structures (DICKSON, 2004).

Thus, advantages for acerola plants resulting from symbiosis with AMF are correlated with the presence of those structures in roots. For example, in the *Arum* type, the symbionts come into close contact through an interface formed as the arbuscules grow. Nutrients absorbed from the soil, especially P and photoassimilated carbon, are transferred through this interface, which is considered as an exchange site for symbiosis. Conversely, in the *Paris* type, intracellular fungal hyphae predominate, spreading from cell to cell within the root cortex and extending to the soil. Those hyphae play a key role in symbiosis because they affect soil nutrient and water absorption by aiding transfer to the plants (MOREIRA & SIQUEIRA, 2006).

Formation and development of AMF morphological structures possibly depends on the plant and fungal species involved and on environmental conditions (ZHAO et al., 2016). Therefore, new research on the occurrence of these morphological structures grown in plant species associated with AMF must be performed. Given the importance of the topic, the present study aimed to assess the morphological pattern of arbuscular

mycorrhizal fungi (AMF) colonization and the soil microbiological activity underlying acerola growth in family farming areas.

MATERIALS AND METHODS

The study area belongs to the municipality of Maranguape – Ceará (CE) and is located at the following geographical coordinates: 3°53'27" South and 34°41'08" West. It has a tropical wet-dry climate (Aw), according to the Köppen climate classification, and the collection period spanned from April to June 2014, with 368mm rainfall and 30°C average temperature during the sampling season (FUNCEME, 2017). Four family farming areas planted with acerola were selected at the study site. Those areas were designated according to plant age as A1-3, A2-3, and A3-3 (3-year-old) and A4-2 (2-year-old), at collection time. The cultural treatments applied in the orchards consisted of soil supplementation with dry cow dung and mineral fertilization using sand. Furthermore, spontaneous vegetation was retained on the soil surface after hoeing and cutting, to maintain soil cover, thereby increasing soil moisture and reducing the erosion processes.

Samples were randomly collected at 20 sites in each area, at 0–20cm depth, using stainless steel hoes and shovels to avoid contaminating the material after collecting each sample. Soil and root samples were packed in sealed bags and labeled according to the name of each study area. The samples were transported to the Soil Microbiology Lab, Department of Soil Sciences, Universidade Federal do Ceará (UFC), and subsequently sieved through a 2mm mesh for the physical, chemical, and microbiological analysis procedures.

A composite sample of the mixture of soils from the 20 sampling points of each area was sent to the UFC Soil and Water Analysis Laboratory for physical and chemical characterization, according to the methods described by EMBRAPA (1997) (Table 1). However, the material for microbiological analysis (arbuscular mycorrhizal colonization, soil basal respiration, and spore density) was stored under refrigeration (4°C) at the UFC Soil Microbiology Laboratory, until the analyses were performed.

Arbuscular mycorrhizal colonization (AMC) was assessed according to a method adapted from KOSKE & GEMMA (1989). For each sample collected, 10 root fragments were arranged in parallel on microscope slides and observed at 400x magnification under an optical microscope. The percentage of root colonization by AMF was quantified using the method described

Table 1 - Physical and chemical attributes of the topsoil layer (0-20cm) collected from four areas of the municipality of Maranguape-CE.

	A1-3	A2-3	A3-3	A4-2
Textural classification	Sandy loam	Sandy loam	Sandy loam	Sandy-clayey loam
pH (water)	7.7	5.6	6.1	5.9
EC (dS m ⁻¹)	0.56	0.31	0.45	0.33
Ca ²⁺ + Mg ²⁺ (cmol _c kg ⁻¹)	6.70	2.60	3.60	3.80
Na ⁺ (cmol _c kg ⁻¹)	0.71	0.50	0.19	0.40
K ⁺ (cmol _c kg ⁻¹)	0.35	0.20	0.19	0.28
H ⁺ + Al ³⁺ (cmol _c kg ⁻¹)	0.83	3.30	2.64	1.82
V (%)	91.0	50.0	62.0	71.0
N (g kg ⁻¹)	0.64	0.40	0.45	0.74
OM (g kg ⁻¹)	10.34	6.83	8.28	12.0
P (mg kg ⁻¹)	81.0	40.0	44.0	24.0

EC: electric conductivity; OM: organic matter; A1-3; A2-3; A3-3 (3-year-old area); A4-2 (2-year-old area).

by MCGONIGLE et al. (1990) under an optical microscope at 400x magnification. The segments with vesicles, arbuscules, or typical hyphae, sometimes attached to spores, were considered colonized, and results were expressed as the percentage of root colonization. Based on the identification of AMF structures observed in the roots after the root staining procedure, the morphological pattern mycorrhization was classified as “*Arum*”, “*Paris*” or “intermediate”, according to DICKSON (2004).

The soil basal respiration (SBR) was assessed by quantifying the carbon dioxide (CO₂) released in the microbial respiration process from 50g of soil packed in an airtight glass container, according to the method described by MENDONÇA & MATOS (2005). AMF spore density (DS) was estimated in 100g soil subsamples of the samples collected at the 20 sampling sites in each orchard by wet sieving (GEDERMANN & NICHOLSON, 1963).

The statistical software package Statistics (SPSS) version 20 was used to perform the descriptive analysis of the study parameters. The following statistics were calculated: minimum, mean, maximum, and standard deviation. Data dispersion around the mean was calculated using the coefficient of variation (CV) and classified according to WILDING & DREES (1983) as follows: low for CV ≤ 15%; moderate for 15% < CV ≤ 35%; and high for CV > 35%. To test the hypothesis of normal distribution, the Shapiro-Wilk test was performed at 5% probability. The study parameters SBR, DS, and AMC of each area were plotted in box plots showing the descriptive statistics data and the presence or absence of outliers.

RESULTS AND DISCUSSION

The AMF morphological pattern as evaluated based on root colonization showed the predominance of *Paris*-type morphological structures (Figure 1) in acerola roots, in addition to the presence of vesicles and intracellular hyphae (Figure 1). Despite the lack of studies on AMF morphological patterns in the acerola crop, results from the present study indicated that this plant species favors *Paris*-type morphologies. However, further studies will be necessary to elucidate the interaction between the symbionts because research on AMF morphological patterns in acerola crops is nonexistent. Morphological pattern of colonization depends on the plant-AMF combination, according to FEDDERMANN et al. (2010). The same authors reported that the plant and AMF genera are not necessarily compatible regarding the morphological pattern of colonization; although, different plant species tend to favor specific colonization morphologies. Furthermore, GUCWA-PRZEPIÓRA et al. (2016) stated that, although, the plant species plays a key role in determining the morphological pattern of AMF root colonization, the type of pattern will depend on the AMF species and on the environmental conditions.

The *Paris* morphological pattern is characterized by the presence of intracellular hyphae and few arbuscules, which are often absent. Thus, the failure to identify arbuscules, as occurred in the presence study, has already been reported in previous studies (SMITH et al., 2009), particularly when the *Paris* morphology is prevalent. However, this absence does not necessarily preclude the main

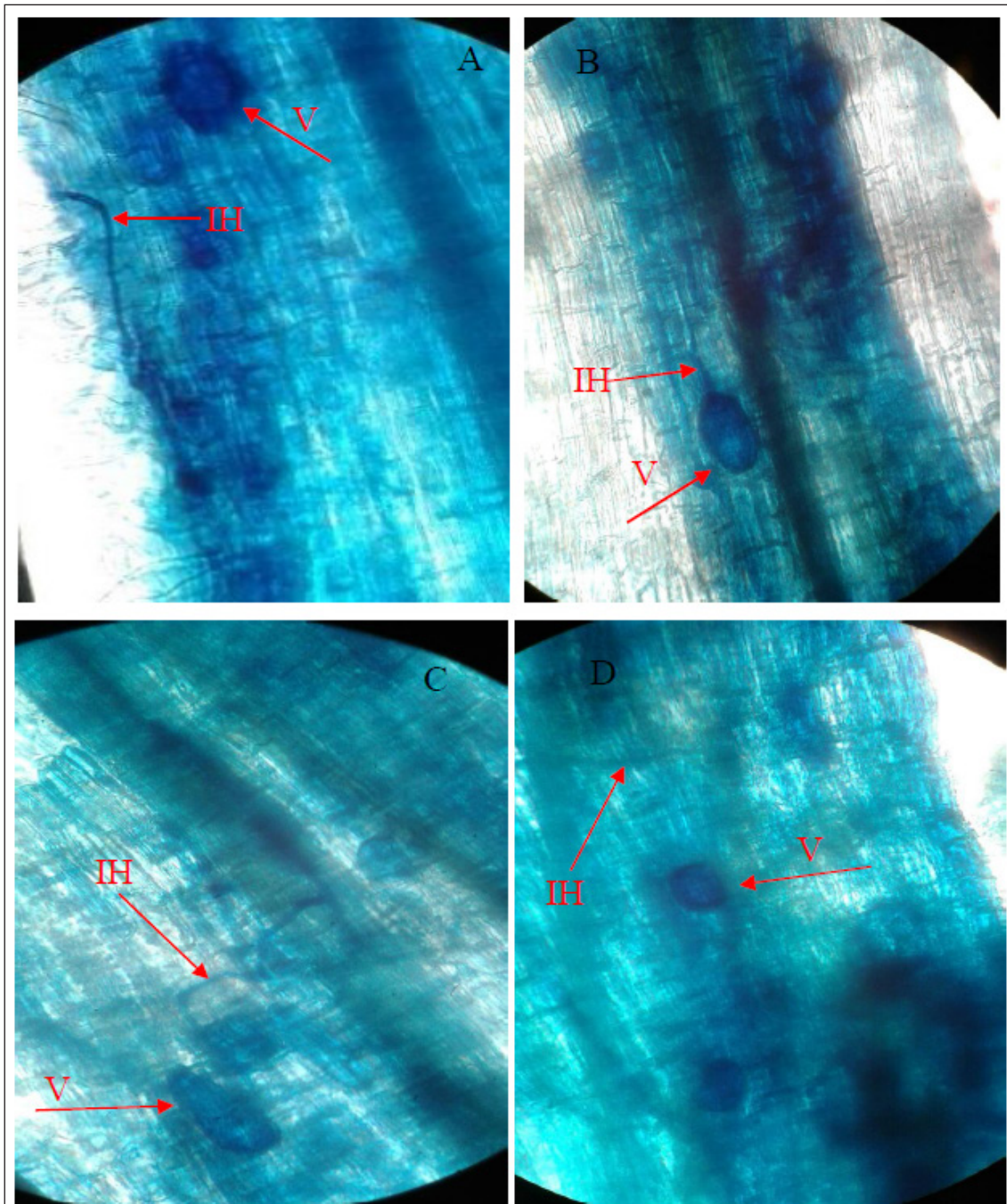


Figure 1 - Photomicrographs of the roots of acerola plants collected in the areas A1-3 (A), A2-3 (B), A3-3 (C), and A4-2 (D) after root staining. V: vesicle; IH: intraradical hyphae.

function of AMF in plant growth, which is the absorption of P, Cu, and Zn, poorly mobile nutrients in soil. SMITH & SMITH (2011a) reported that intracellular hyphae, arbuscules, and structures with intermediate morphology are involved in the

transfer of nutrients to the host plant, not arbuscules alone. Therefore, the presence of the *Paris* morphological pattern in acerola roots is a positive factor for nutrient absorption in the crop, enhancing plant growth, which was also noted in other plant

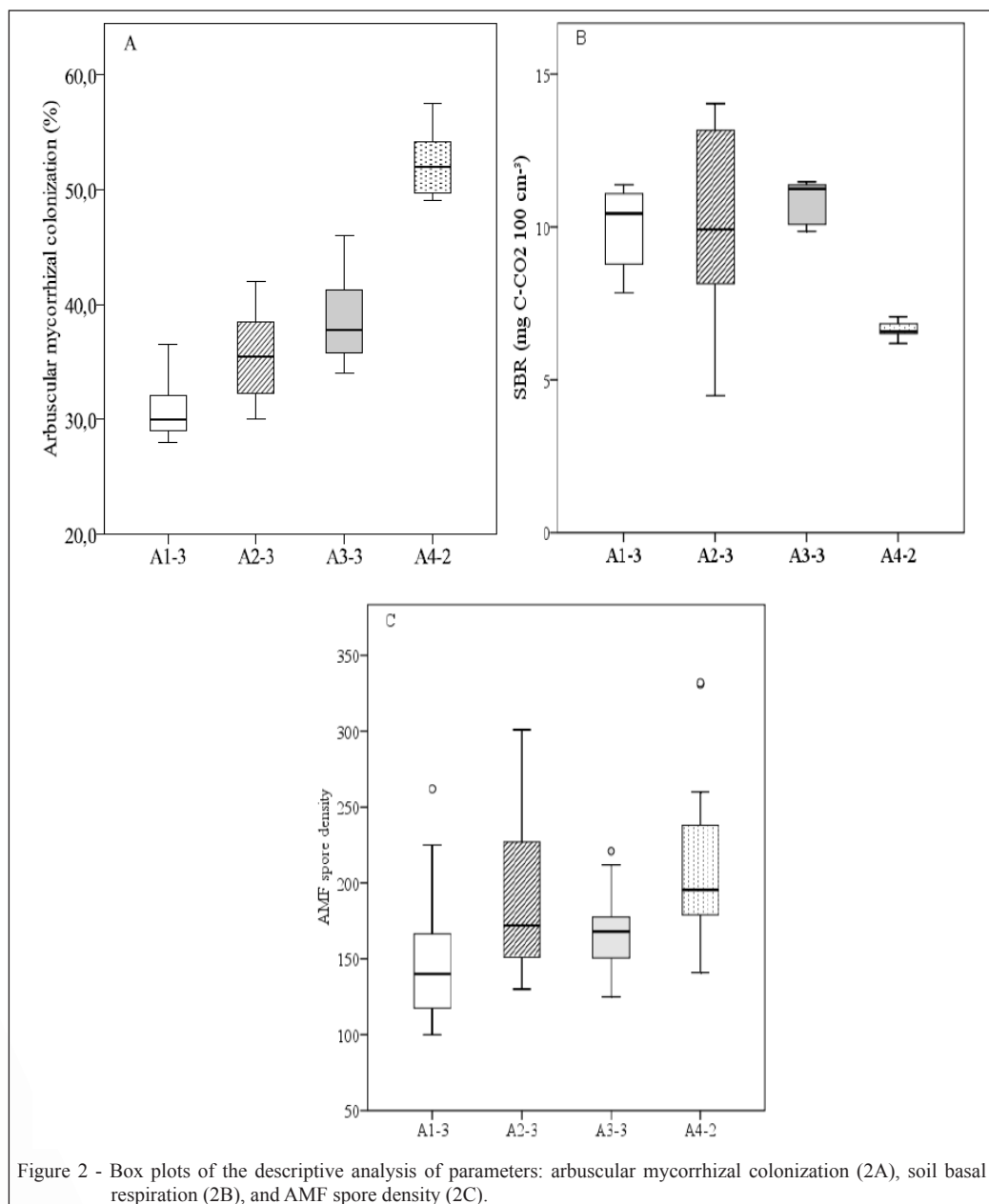
species in a study conducted by BREARLEY et al. (2016). Similar findings were also reported by HARIKUMAR et al. (2015) in plants with the *Paris* morphological pattern with satisfactory growth.

The acerola AMC and DS data failed to follow a normal distribution, according to the Shapiro-Wilk Test ($P \leq 0.05$); although, the SBR data tended toward a normal distribution.

The box plots (Figure 2) showed the parameters assessed in the four areas analyzed in the municipality of Maranguape – CE. Data dispersion

around the mean, expressed as coefficient of variation (CV), was low ($CV \leq 15\%$) for the AMC and SBR variables, excluding the area A2-3 for the latter. DS showed a moderate CV ($15\% < CV \leq 35\%$) in all areas except A3-3.

The AMC of acerola ranged from 30.85% in area A1-3 to 52.17% in area A4-2 (Figure 2A). The above values were affected by the P contents reported in each area, which were 81 and 24 mg kg^{-1} , respectively (Table 1), decreasing the percentage of colonization in area A1-3 and



markedly increasing the colonization in area A4-2 because AMC may be adversely affected by soil P concentrations. High soil P content directly decreases the mycorrhizal colonization of several plant species, including acerola, according to YANG et al. (2016), DANTAS et al. (2015), and TAFFOUO et al. (2014). SMITH & SMITH (2011b) reported that plants in good nutritional conditions trigger mechanisms to reduce AMF colonization toward reducing the energy cost of maintaining the fungus in the plant species.

SBR showed similar values in the four study areas, ranging from 6.64 to 10.81 mg C-CO₂ day⁻¹ 100 cm⁻³ soil in areas A4-2 and A3-3, respectively (Figure 2B). The lower SBR value observed in area A4-2 is related to the shorter crop growth season, because the soil microbial community is still presumably stabilizing from the stress of growing the acerola orchard. The traffic of machines and agricultural implements for soil plowing, harrowing, liming, or mineral fertilization, which are soil management practices that cause stress to the microbial population, is constant at the early growth season of perennial crops. PRAGANA et al. (2012) confirmed that the constant soil disturbance and absence of soil cover in conventional systems may reduce the soil microbial population and, therefore, microbial activity, requiring time to reestablish the balance reported prior to the stress on the organisms.

The DS of areas A1-3 and A4-2 was 150 and 211 spores per 100 g soil, respectively (Figure 2C). This marked variation in DS is mainly related to the climatic condition, soil, rainfall, and cultural treatments applied to the study areas. MELLONI et al. (2016) confirmed that DS usually shows a marked variation in cultivated soils because AMF spores are affected by several biotic and abiotic factors. LIMA et al. (2013) and JIMÉNEZ-LEYVA et al. (2017) confirmed that the DS in the rhizosphere is variable and is related to root distribution, morphology, and age, in addition to other factors including soil chemical characteristics (particularly P content), regional rainfall, and AMF species.

Thus, the decreased DS value observed in the A1-3 area was related to the high soil P content (Table 1), and the increased spore count was related to the decreased P content in the soil of area A4-2 (Table 1). These results are primarily related to the soil P concentrations because they may negatively or positively affect the signaling and growth of AMF in the soil and host plant.

CONCLUSION

The morphological pattern of the *Paris* type was prevalent in the acerola root system, clearly showing a crop affinity for that pattern. Although, the need for further studies to better understand the presence of that pattern in roots should be emphasized because research on acerola in this area of knowledge in soil biology is nonexistent.

The study season and acerola orchard management in the family farming areas decreased the basal soil respiration. The soil P content may have adversely affected AMF root colonization and spore density.

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