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Effect of arbuscular mycorrhizal fungi on survival and growth of micropropagated *Comanthera mucugensis* spp. *mucugensis* (Eriocaulaceae)

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The use of micropropagation technique has been an alternative to conservation of endangered species, *Comanthera mucugensis* subsp. *mucugensis* (popular namely sempre viva de Mucuge); however, there is no information on the effect of arbuscular mycorrhizal fungi (AMF) on the acclimation process of micropropagated plants. This study evaluated the survival, growth and nutritional aspects of the species, *C. mucugensis* subsp. *mucugensis* inoculated with native AMFs in greenhouse condition. The design of the experiment consisted initially of 80 sampling units divided into four treatments: plants inoculated with native AMF, with microbiota filtrate from soil, with AMF plus filtrate and control (non-inoculated plants). At three and eleven-month-old, the plants were collected for evaluation of growth, nutrition and mycorrhizal colonization. After eleven months of experiment, survival rate of AMF and AMF plus filtrate plants were 62.5 and 87.5%, respectively, and only one microbiota filtrate and one control plants survived. AMF inoculation also provided increase in n dry matter of rosettes and permitted obtaining flowering ten-month-growth plants. Rates of mycorrhizal colonization were high at three (aproximately 64.9%) and eleven (aproximately 94.5%) months for AMF and AMF plus filtrate plants. Number of spores in rhizosphere soil of mycorrhizal plants was also high (1599 per 100 dm^3 of soil) and seven diferent species of AMF were identified at the end of experiment. Data set evidenced mycortrophic character of *C. mucugensis* subsp. *mucugensis* and the importance of AMF inoculation for acclimation and survival of microprogagated plants which is essential for conservation of this endangered plant.

Key words: Micropropagation, nutrition, arbuscular mycorrhiza fungi, sempre viva de Mucuge, aclimatation.

INTRODUCTION

The Eriocaulaceae family comprises eleven genera and ca. 1200 species, has a pantropical distribution (Echternacht et al., 2010), and presents its diversity center on the Espinhaço Range between Minas Gerais and Bahia (Giulietti and Hensold, 1990; Sano, 2004). About 70% of the total Brazilian species of Eriocaulaceae

occur at Espinhaço Range, 85% are endemic and often are restricted to a single mountain (Giulietti et al., 2005; Costa et al., 2008). The species, *Comanthera mucugensis* subsp. *mucugensis* is one of this microendemic Eriocaulaceae plants that occured on municipality of Mucuge (Bahia) at eastern side of the Chapada Diamantina region. This species is popularly known as sempre viva de Mucuge (evergreen of Mucuge) and its inflorescence remains with the same color and shape when their scapes, chapters and flowers are collected for making dried floral arrangements. At region of rupestrian field on Mucuge where these plants occur naturally, they were one of the main sources of income for local inhabitants at the mid-twentieth century, and each year were sold tons of flowers, especially to Europe and the United States (MMA/PNMA, 1996), whichreduced the natural population since the flowers are still at anthesis when collected to be sold as ornamental (Giulietti et al., 1988; Cerqueira et al., 2008).

Recently, *C. mucugensis* subsp. *mucugensis* was prohibited from being collected because their exploitation has been carried out without planning and without any control or cultivation (Lima-Brito et al., 2016), and currently, this plant is on the Official List of Species of the Brazilian Flora Endangered (MMA, 2008). Some tentatives of plant management are already being developed at Parque Municipal de Mucuge, as to protect *C. mucugensis* subsp. *mucugensis* populations and promote the propagation and cultivation, seeking alternative sources of income to the population of the municipality (Paixão-Santos et al., 2003; Ramos et al., 2005; Teixeira and Linsker 2005).

With the aim to increase *C. mucugensis* subsp. *mucugensis* populations in Mucuge region, the micropropagation technique has been used as a viable option for the production of seedlings of this species (Lima-Brito et al., 2011; Pêgo et al., 2013). Despite the advantages in using this technique, there are still some obstacles to their wide application, especially as regards acclimation, that is, the conditions to be transplanted *in vitro* to greenhouse, since mortality rate of *C. mucugensis* subsp. *mucugensis* micropropagated plants is high.

The absence of beneficial soil microorganisms can result to negative effects on the plant acclimation process due low adaptation to new environmental conditions imposed (Borkowska, 2002). Studies on the association of arbuscular mycorrhizal fungi (AMF) with some agronomic and ornamental plants demonstrate benefits of these microorganisms as plant growth regulators and their importance to management and acclimation (Rocha et al., 2006; Yadav et al., 2013; Moreira et al., 2015; Villarreal et al., 2016). Arbuscular mycorrhizal fungi (AMF) are an important microbial group of the soil, which form a mutualistic symbiosis with the roots of plants affecting several processes and functions in the ecosystem such as nutrient cycling, plant productivity and competition (Hazard et al., 2013). This microorganism have been used as an alternative to increase the resilience of many species during the acclimation process, stimulating the autotrophic stage of transition from *in vitro* to soil and influencing morphogenesis and architecture of root, ensuring a health formation and development of root system after transplanting (Zemke et al., 2003; Kapoor et al., 2008; Stancato and Silveira, 2010). Apart from this, AMF can act as biological controller of some pathogens and to reduce tensions as nutrition, availability of water and salinity involved on micropropagation (Schubert et al., 1990; Jaizme-Vega and Azcón, 1991).

In the present study, the authors evaluated native AMF and microbiota inoculation on acclimation of *C. mucugensis* subsp. *mucugensis* micropropagated plants, analysing survival and nutritional status with goal to contribute to process of population restoration of this endangered plant.

MATERIALS AND METHODS

In vitro culture

In the experiment, 120 days old micropropagated plants of the *C. mucugensis* subsp. *mucugensis* obtained from the Vegetable Tissue Culture Laboratory of the Horto Florestal Experimental Unit, belonging to the Biological Sciences Department of the Feira de Santana State University, in the municipality of Feira of Santana, Bahia were used. The chemical characterization of the *in vitro* plants was carried out at the Laboratory of Analysis of Vegetable Tissues of the Cocoa Research Center (CEPEC) of the Executive Committee for Cocoa Plantation Planning (CEPLAC). The results were: N = 42.18 $g.Kg^{-1}$; P = 1.98 $g.Kg^{-1}$; K = 22.92 $g.Kg^{-1}$; Ca = 2.29 $g.Kg^{-1}$; Mg = 1.28 $g.Kg^{-1}$; Cu = 2.33 $mg.Kg^{-1}$; Fe = 38.12 $mg.Kg^{-1}$; Mn = 44.3 $mg.Kg^{-1}$; Zn = 46.84 $mg.Kg^{-1}$.

Obtaining plant material

The experiment was conducted in a greenhouse at the University of Santa Cruz (Ilheus, BA) under natural conditions of temperature and luminosity. Micropropagated seedlings of *C. mucugensis* (Giul.) L.R.Parra & Giul. subsp. *mucugensis* were provided by the Tissue Culture Laboratory of the State University of Feira de Santana (UEFS), and grown in plastic pots containing 0.4 dm^3 of soil collected at rupestrian field on Parque Municipal de Mucugê (Mucugê, Bahia, Brazil; 12°59'27''S, 41°20'11''W and 980 a.s.l). This native soil was previously sterilized at 121°C for two cycles of 1 h with 48 h interval, and after reaching ambient temperature, the resulting pH (measured in water) was 2.8 and it was not adjusted. Previous experiments liming on soil and substrate (coarse and fine

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sand) indicated that this plant do not tolerate (die) soil pH reaching 5.

The soil of rupestrian field collected for the experiment presented a sandy texture class with 84.80% sand, 15.06% silt and 0.14% clay. The chemical characterization of the soil was performed by the Chemical Analysis Laboratory of the Department of Soil Science College of Agriculture "Luiz de Queiroz", University of São Paulo (USP-ESALQ) following Raij et al. (2001) method and presented the following results: pH, 2.7 (in $CaCl_2$); organic matter, 76 g dm^{-3}; P, 5 mg dm^{-3}; S, 2 mg dm^{-3}; K, 0.4 $mmol_c$ dm^{-3}; Ca, 2 $mmol_c$ dm^{-3}; Mg, 2 $mmol_c$ dm^{-3}; Al, 19 $mmol_c$ dm^{-3}; H + Al, 386 $mmol_c$ dm^{-3}; Cu, 0.1 mg dm^{-3}; Mn, 1.1 mg dm^{-3}; Fe, 30 mg dm^{-3}; Zn, 1.8 mg dm^{-3}.

Ten plants of *C. mucugensis* subsp. *mucugensis* were collected on field (from natural population at Mucuge) and evaluated for nutrient composition aiming to prescribe a nutritional fertilization previously to perform the experiment. Dry matter of the rosettes (leaves) was chemical characterized (Raij et al., 2001) and results were: N, 10.78; P, 0.27; K, 1.53; Ca, 0.55; Mg, 1.40; S 0.76 (all maconutrients at g kg^{-1}); B, 4.67; Cu, 0.80; Fe, 26.20; Mn, 8.10; Zn, 4.60 (all micronutrients at mg kg^{-1}).

Experimental design

The experimental design was completely randomized and initially 80 sampling units divided among the control and three treatments: plants inoculated with native AMF, with microbiota filtrate from soil, with AMF plus microbiota filtrate. In 20 replicates from each treatment, 12 were collected at three month plant growth to investigate the initial mycorrhiza establishment at the acclimatization phase. The remaining eight plants were collected at 11 month of plant growth. The spores of native AMF used as inoculum were obtained from the multiplication pot using *C. mucugensis* subsp. *mucugensis* as host plant, since there was low sporulation on the previous attempt using a conventional host plant (*Brachiaria decumbes*).

Spores were isolated from 100 g of soil using the technique of wet sieving of Gerdemann and Nicolson (1963) and centrifugation in 50% sucrose using the technique of Jenkins (1964). To simulate the natural microbial composition of soil, a filtrate was prepared using a suspension of field soil with autoclaved distilled water (1:10 m/v), which was stirred for 24 h (Sudová and Vosátka, 2008). Subsequently, the material was passed through a glass funnel containing filter paper (Whatman no. 1) with the aid of a vacuum pump retaining the solid part and mycorrhizal propagules.

After transplantation from *in vitro* condition to the platic pots in a greenhouse, the micropropagated plants (85 days old), and according to the treatment, received 10 ml suspension containing: mycorrhizal inoculum with 470 spores, microbiota filtrate and mycorrhizal inoculum and filtrate.

Fertilization of plants in pots

Every week, the plants were irrigated at intervals of 48 h with 30 mL of ¼ ionic strength nutrient solution adapted from Hoagland and Arno (1950). The irrigation with distilled water of the same volume was interspersed with nutrient solution. The complete nutrient solution (in mg L^{-1}) consisted of: N, 70.00; P, 5; K, 45.36; Ca, 50; Mg, 12.16; S, 64.00; Zn, 0.01; B, 0.11; Cu, 0.005; Fe, 0.25; Mn, 0.11; Mo, 0.002 as H_3BO_3; $MnSO_4$; $ZnSO_4$; $CuSO_4.5H_2O$; $(NH_4)_6Mo_7O_{24}.4H_2O$; Fe-EDTA; KH_2PO_4; $(NH_4)_2SO_4$; K_2SO_4; $Ca(NO_3)_2$; $MgSO_4$ salts.

Dry biomass and nutritional analysis

For the analysis of biomass, rosettes (leaves) were dried at 60°C in an oven with forced air circulation until constant weight. Dry matter was obtained and due to the small volume of plant material, only one sample (the sum of all replicates) per treatment was sent to the Laboratory of Mineral Nutrition of Plants USP - ESALQ for nutritional analysis. The methodologies used in this analysis were: P: colorimetry (ammonium metavanadate method), S: colorimetry (turbidimetric barium sulfate), K, Ca and Mg by atomic absorption spectrophotometry, Cu, Fe, Mn and Zn: absorption spectrophotometry atomic; sulfuric digestion for total N, B: colorimetry (Azomethine H method).

Assessment of AMFs colonization

To estimate the percentage of mycorrhizal colonization, *C. mucugensis* subsp. *mucugensis* roots were blenched in 10% KOH and stained using trypan blue according to the methodology described by Phillips and Hayman (1970). The estimate of colonization of root segments was based on the method of intersection enlarged (McGonigle et al., 1990).

Extraction and quantification of spore production

Spores of rhizosphere soil samples were extracted following the technique of decanting and wet sieving of Gerdemann and Nicolson (1963) combined with the technique of centrifugation in sucrose solution at 50% of Jenkins (1964). The isolated spores were quantified in a Petri dish and stored in tubes, kept in the refrigerator until analysis of taxonomic characteristics needed for identification.

Taxonomic identification of AMFs

The spores were previously isolated in separate groups of morphotypes under a stereomicroscope and then mounted on slides with permanent PVLG resin and Melzer reagent (Morton et al., 1996). Spores preserved on slides were observed under an optical microscope (magnification of 1000x) and morphological characters such as size (in µm), shape, color, structure and decoration of wall, type of hyphae and spore germination mode, were recorded for comparison with the related literature. The identification was carried out by using Schenck and Perez (1988) manual and current avaliable literature.

Statistical data analysis

The data obtained for rosette dry mass, spore number and percentage of mycorrhizal colonization were compared by a one-way ANOVA/Tukey multiple comparison or a t-test when appropriate. The analyzes were performed in the statistical package STATISTICA 8.0 (Statsoft 2002).

RESULTS

Of the total 12 sample units for each treatment collected after three months (Figure 1A, B and C) of growth in greenhouse, 100% of *C. mucugensis* subsp. *mucugensis* plants inoculated with native AMF and inoculated with AMF plus microbiota filtrate survived. Three plants from microbiota filtrate treatment and four from control died. At nine months of growth plants initiate scape (flowering) production (Figure 1D) and some flowers were obtained at the end of eleven month of growth at greenhouse

Figure 1. Partial view of experiment and mycorrhizal colonization in roots of an *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF. (A) Partial view of experiment with *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF after three months of growth in greenhouse. (B) Partial view of experiment showing plants with their floral scapes (arrows) developed after eleven months of growth. (C) Detail of a rosette from an AMF inoculated plant with three months of growth. (D) Detail of a rosette from an AMF inoculated plant with some initial flower scapes developing (nine months of growth in greenhouse).

(Figure 1B). At the end of the experiment from the eight remaining sampling units, five plants from mycorrhiza treatment and seven plants from mycorrhiza plus filtrate treatment survived. On the other hand, seven plants from filtrate treatment and seven plants from control died.

Aboveground biomass

Rosette dry mass from three month growth plants of *C. mucugensis* subsp. *mucugensis* presented significant differences ($p \leq 0.05$) among mycorrhiza treatments and non mycorrhizal (control and microbiota filtrate) plants evidenced the strong influence of AMF on biomass production (Table 1). The mean values of rosette biomass of eleven months growth plants from AMF inoculated and AMF plus microbiota filtrate did not statistically differ (*t* test $p \leq 0.05$) because only one plant from control and microbiota filtrate treatments survived; statistical analysis was not carried out, but the diference from mycorrhiza treatments was evident (Table 1).

Mycorrhizal colonization

The mean values of mycorrhizal colonization in AMF inoculated and AMF plus filtrate plants did not differ significantly from each other in both collection times; however, there was an increase in the percentage of colonization of these two treatments when comparing the three and eleven months palnt (Table 1). AMF inoculated plants showed the highest percentages of colonization in root fragments of plants evaluated at three and eleven months of growth. In roots of non-inoculated control and microbiota filtrate inoculated plants, no signal of mycorrhizal structures were observed in both periods (Table 1). During qualitative evaluation with microscope, intraradical hyphae (Figure 2A) and vesicles (Figure 2B) were observed, however arbuscules were the structures more frequently observed (Figure 2C and D).

Nutritional diagnosis

The levels of macro and micronutrients observed in composed samples of rosette dry biomass *C. mucugensis* subsp. *mucugensis* at three and eleven months of growth are presented in Table 2. The yield of dry matter of filtrate and control plants in eleven months old plants was insufficient for chemical analysis, therefore are not presented in Table 2. In general, there was no large variation on nutrient levels among plants from

Table 1. Biomass of rosettes and mycorrhiza of *C. mucugensis* subsp. *mucugensis* micropropagated plants inoculated with native AMF, microbiota filtrate, AMF plus microbiota filtrate and control plants after three and eleven months of growth in greenhouse conditions.

Plant growth (months)	Parameter	Treatment			
		Control	AMF	Microbiota filtrate	AMF plus Microbiota filtrate
Three	Rosette (leaves) dry weight (mg)	82±18b	145±28a	80±14b	137±18[a]
	Mycorrhiza colonization (%)	0	67.7±12.8	0	62.1±13.8
	Number of spores (per 100 g of soil)	182±31	1118±212	36±14	1025±116
Eleven	Rosette (leaves) dry wieght (mg)	280	616±117	310	491±111
	Mycorrhiza colonization (%)	0	94±5.89	0	95±3.46
	Number of spores (per 100 g of soil)	190	1749±306	25	1449±74

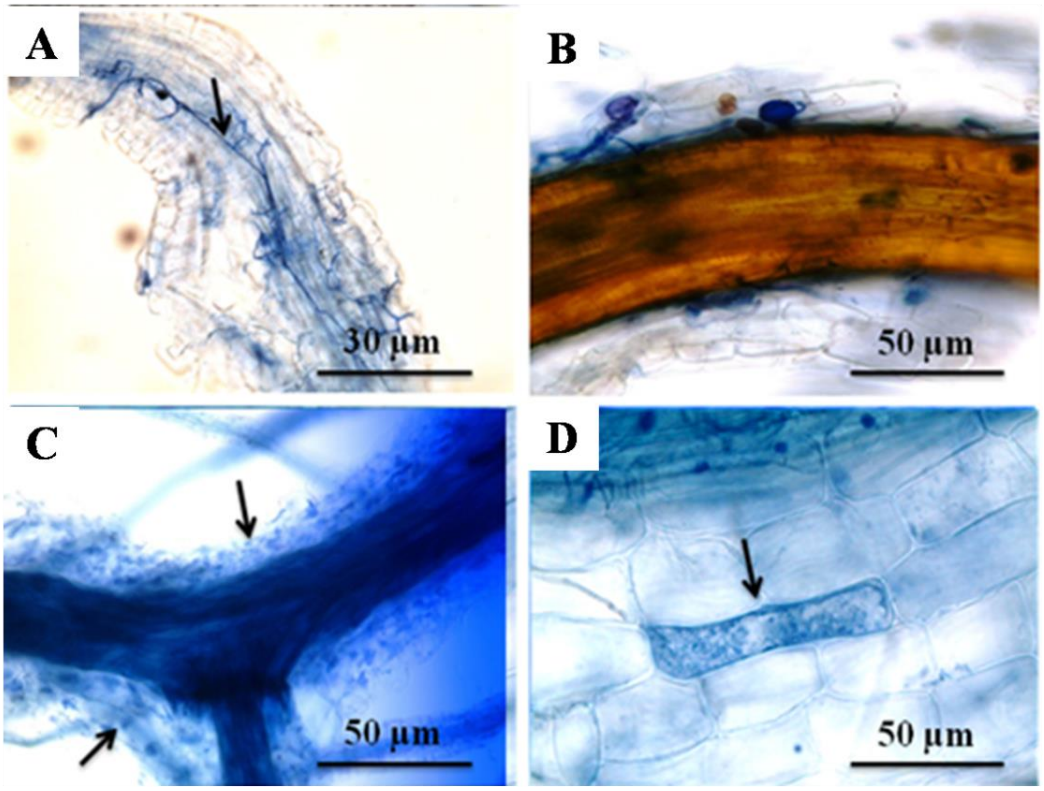

Figure 2. Partial view of experiment and mycorrhizal colonization in roots of an *C. mucugensis* subsp. *mucugensis* plants inoculated with AMF. (A) Mycorrhizal colonization in roots of *C. mucugensis* subsp. *mucugensis* AMF plus microbiota filtrate inoculated plants. Arrow indicate a extraradical hypha; (B) detail of some vesicles in the cortex of an AMF plus microbiota filtrate plant; (C) general view of a densely arbuscules occupied cortical cells (arrows); (D) detail of an arbuscule (arrow) in the cortical cell of an AMF inoculated *C. mucugensis* subsp. *mucugensis* root segment.

differet treatments.

Quantification of spores

The evaluation of the number of AMF spores of soil rhizosphere demonstrated, as expected, mycorrhiza and mycorrhiza plus microbiota filtrate plants presented significat differences when compared with filtrate and control plants, but not significately different between them (Table 1).

Quantification performed for eleven month old plants presented mean values not statiticaly different between filtrate plus mycorrhiza and mycorrhiza plants (Table 1).

Table 2. Macronutrients and micronutrients concentrations in rosettes (leaves) of *C. mucugensis* subsp. *mucugensis* with native AMF, microbiota filtrate, AMF plus microbiota filtrate and control plants after three and eleven months of growth in greenhouse conditions.

Plant (months)	Treatment	Macronutrient (g Kg^{-1})						Micronutrient (mg Kg^{-1})			
		N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
Three	Control	32.16	3.82	58.91	2.10	2.10	3.96	19.11	85.70	142.10	61.40
	AMF	34.02	4.06	17.34	2.10	1.80	3.73	18.25	73.70	125.20	67.80
	Microbiota filtrate	36.75	3.85	28.05	2.05	1.90	3.56	17.22	76.80	132.00	53.70
	AMF plus Microbiota filtrate	33.96	4.03	27.03	2.30	2.20	4.15	20.66	76.20	114.30	55.00
Eleven	AMF	35.15	3.66	11.47	2.91	3.0	6.80	3.80	89.8	109.1	49.9
	AMF plus Microbiota filtrated	34.00	3.60	10.90	2.46	3.1	6.6	3.71	100.8	94.1	45.8

Statistical analysis was not performed on the control and filtrate plants due to death of plants.

Taxonomic identification of AMFs

Spores isolated from rhizosphere soil from plants of *C. mucugensis* subsp. *mucugensis* inoculated with mycorrhizae and mycorrhiza plus filtrate used to identify seven species of AMFs listed below:

1. *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler
2. *Glomus macrocarpum* Tulasne & Tulasne
3. *Glomus microaggregatum* Koske, Gemma & Olexia
4. *Glomus microcarpum* Tulasne & Tulasne
5. *Glomus* sp.
6. *Scutellospora dispurpurascens* J.B.Morton & Koske
7. *Scutellospora spiniosissima* C.Walker & Cuenca

The *Claroideoglomus etunicatum* and *Glomus macrocarpon* species were the only species found in both treatments. These spores are shown in Figure 3.

DISCUSSION

High rates of root colonization by native AMF was observed in *C. mucugensis* subsp. *mucugensis* micropropagated plants. These rates influenced growth responses of plants and showed the mycorrhizal dependence (mycotrophism) of *C. mucugensis* subsp. *mucugensis* since non-inoculated AMF plants, even with frequent nutrient solution fertilization on the natural soil, did not grow but died. Our results clearly pointed that *C. mucugensis* subsp. *mucugensis* is a mycotrophic plant with rate of mycorrhizal colonization of eleven old months higher than those observed by Pagano and Scotti (2009) on *Paepalanthus bromelioides* and Aristizabal et al. (2004) in roots of *Paepalanthus* sp., two Eriocaulaceae species. This rate of colonization by AMFs is also seen in other studies with plants of semi-arid environments (with low water availability) which showed a high symbiotic effectiveness between AMF and plant species (Yamato et al., 2008; Estrada et al., 2013).

The effectiveness of the symbiosis between the micropropagated plants of *C. mucugensis* subsp. *mucugensis* and native AMFs was also verified by the production of extensive arbuscules, hypha and spores (completing life cycle of the fungus). Spore density in soil of three-month-old mycorrhized plants of *C. mucugensis* subsp. *mucugensis* growth at greenhouse was similar to those observed by Borba and Amorim (2007) in rhizosphere soil (1014 spores 100 g $^{-1}$ soil) from natural plant population of same plant collected in Mucuge. Number of spores observed in mycorrhizal plants of *C. mucugensis* subsp. *mucugensis* can be considered high, demonstrating the dependence of this plant species on AMF for their development. Pagano and Scotti (2009) studying *Paepalanthus bromelioides* reported 139 spores per 100 g of rhizosphere sandy soil collected from field.

It was possible to isolate and identify seven species of native AMF from mycorrhizal plants of *C. mucugensis* subsp. *mucugensis*, and with the exception of *Scutellospora spiniosissima*, all other AMF identified were reported in massive study of Carvalho et al. (2012) that identified and listed 49 species of AMFs collected in rupestrian field of Minas Gerais.

Nutrient analyses of *C. mucugenis* var. *mucugensis* rosette demonstrated that mycorrhizal plants presented concentration of macro and micronutrients similar to those non-AMF inoculated plants, despite markedly difference in the plant growth. As known, probably, this is the first report on nutrient staus of a Eriocaulaceae plant, so, it is difficult to compare nutrients concentration on leaves of *C. mucugenis* var. *mucugensis* micropropaged plants with other poales plants for example. When we compare leaf nutrients between plants collected on field and from greenhouse experiment, it is observed that concentration of some nutrients such as N and P were higher (three-fold and ten-fold, respectively) in greenhouse plants than field collected plants due to frequent irrigation with nutrient solution.

The presence of DSF in the roots of *C. mucugensis* subsp. *mucugensis* observed in AMF treatments possibly occurred during inoculation, the same being adhered to

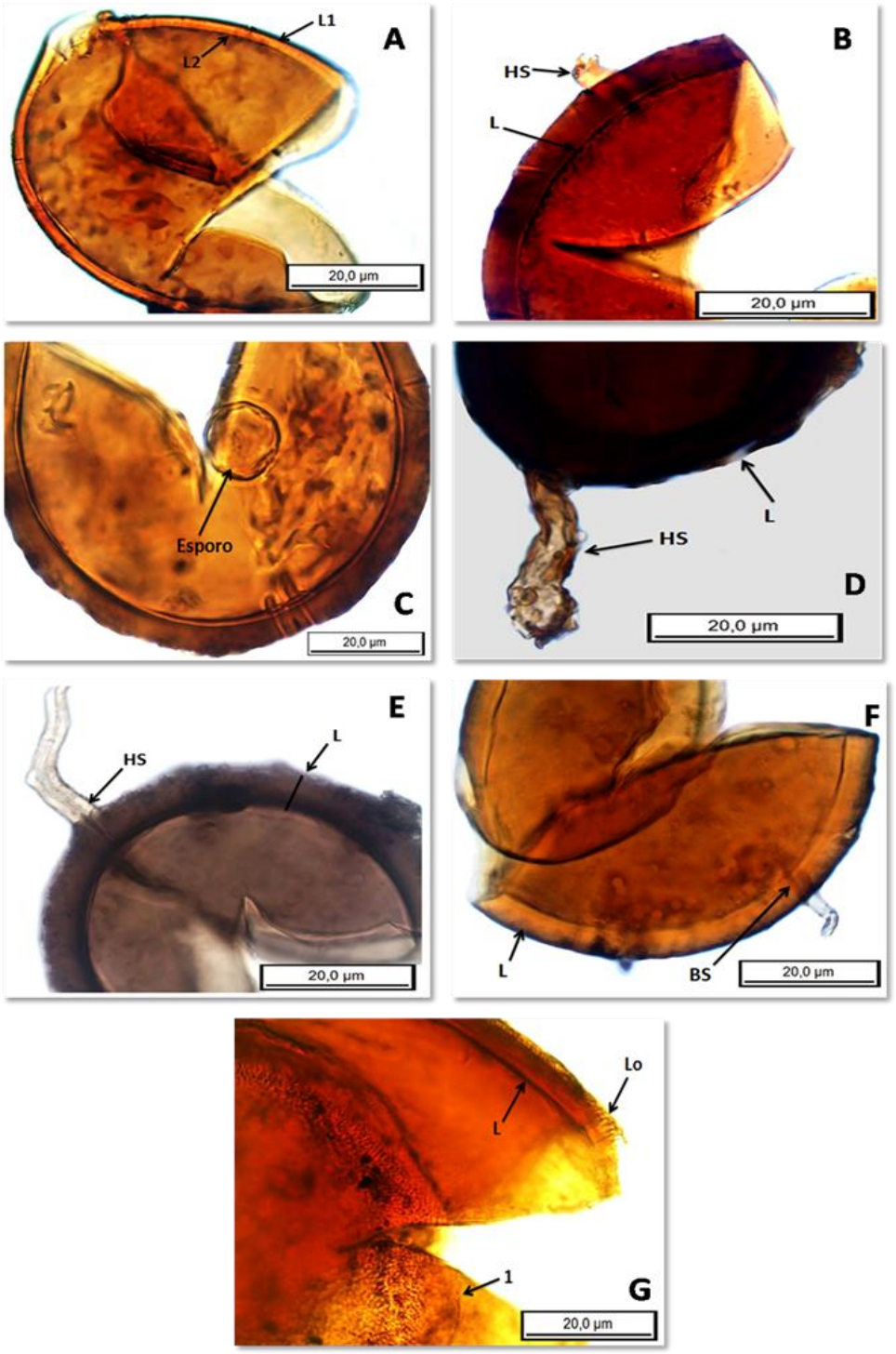

Figure 3. Morphological characterization of AMF spores. (A) Photo of the spore of the species, *Glomus etunicatum* found in the soils of the treatment M and M + F; (B) Image of the spore of the *Glomus macrocarpum* species found in soils of both treatments, M and M + F; (C) Photo of the characteristic spore of the species *Glomus microaggregatum*, found in the soil of the M + F treatment; (D) Photo of the spore of the *Glomus microcarpum* species found in the treatment soil M; (E) Photo of the spore of the species *Glomus* sp. found in the soil of the M + F treatment; (F) Photo of the spore of the species *Scutellospora dispurpurescens* found in the treatment soil M; (G) Photo of the spore of the species *Scutellospora spiniosissima* found in the soil of the treatment M. L, wall layer; Lo, ornamental layer; HS, support hyphae; BS, suspensoroid bulb; 1, illustration of the wall layer; Seta, characteristic structure of the species.

AMFs spores were isolated from soil samples. Reports of the coexistence of DSFs and AMFs in the roots of plants stressed environments (arid environments, acidic and nutrient-poor soils) have become increasingly common in studies involving symbiotic associations with fungi (Lingfei et al., 2005; Porras-Alfero et al., 2008; Schmidt et al., 2008).

The filtrate of soil microorganisms combined with the native AMFs also had favorable responses on survival and acquisition of dry matter of micropropagated *C. mucugensis* subsp. *mucugensis* plants. However, when inoculated alone, microbiota filtrate did not promote plant growth and reduced the plant survival as observed in the control plants. The influence of soil microbiota on plant development as well as possible interactions between the microbial communities present in the rhizosphere and their consequent contribution to plant productivity are widely discussed in the literature (Walker et al., 2003; Artursson et al., 2006; Bonfante and Anca, 2009; Smith and Smith, 2011).

Native AMFs inoculated in *C. mucugenis* var. *mucugensis* were essential for plants survival and growth, permitting the acclimatization at greenhouse on natural soil. The establishment of *in vitro* grown seedlings in soil is hampered by weak root system at the beginning of acclimation, however, the symbiotic association between AMF and plant roots increases the survival rate of plant to strengthen the root system (Yadav et al., 2012). This strengthening can reflect the importance of AMF for nutrients and water uptake at low fertilized environments, defense against pathogens, decreased water stress improving some important characteristics for plant acclimation (Joshee et al., 2007; Pindi, 2011; Singh et al., 2012; Yadav et al., 2013).

In this study, a relatively high amount of organic matter was observed in the soil (76 g dm^{-3}), one of soil characteristic that may have influenced the number of AMF species found. Borba and Amorim (2007) justified the increased number of species of mycorrhizal fungi in the rhizosphere soil, possibly due to a greater accumulation of soil organic matter. Moreover, the species richness from the rhizosphere soil of potted *C. mucugensis* subsp. *mucugensis* may have been influenced by soil type and growing conditions. According to Carvalho (2012), the high diversity of AMF on rupestrian fields can be explained by the heterogeneity of habitats in this environment and the occurrence of AMF species influenced by soil physical properties and also tolerance of these species to low humidity, as shown in some quantitative studies (Conceição and Pirani, 2005).

Conclusion

In this study, the authors reported on native AMF populations inoculated on *C. mucugensis subsp. mucugensis* plants, but the influence of one determined fungi species was not tested and is a subsequent step to evaluate the influence of mycorrhiza inoculation. The study shows that AMF inoculation is undoubtedly an important biotechnological tool and encourages the use of these microorganisms in conservation programs of endangered *C. mucugensis subsp. mucugensis*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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