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Production of native arbuscular mycorrhizal fungi inoculum under different environmental conditions

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ABSTRACT

In order to obtain an arbuscular mycorrhizal fungi (AMF) native inoculum from *Sierra de Moa* and determine the most appropriate conditions for its big scale production, four light and temperature combinations were tested in three plant species (*Calophyllum antillanum*, *Taliparititi elatum* and *Paspalum notatum*). Growth and development parameters, as well as the mycorrhizal functioning of the seedlings were evaluated. The natural light treatment under high temperatures (L-H) was the most suitable for the growth and development of the three plant species, showing the highest total biomass values, mainly of root, and a positive root-shoot ratio balance. This treatment also promoted higher values of root mycorrhizal colonization, external mycelium and AMF spore density. A total of 38 AMF species were identified among the plants and environmental conditions tested. *Archaeospora* sp.1, *Glomus* sp.5, *Glomus brohultii* and *G. glomerulatum* were observed in all the treatments. The L-H condition can be recommended for native inoculum production, as it promotes a better expression of the AM symbiosis and an elevated production of mycorrhizal propagules.

Introduction

Arbuscular mycorrhizal fungi (AMF) (*Phylum Glomeromycota*) are present in almost all the terrestrial ecosystems.[1] They associate with the roots of more than 80% of the vascular plants, giving place to a mutual symbiosis denominated arbuscular mycorrhiza (AM).[2]

The AM enhances the absorption of water and nutrients, mainly P.[1] It also increases the tolerance of plants to biotic and abiotic stresses, as pathogens, drought and high salinity.[3,4] Besides that, the AM plays a critical role in the functional and successional processes of plant communities as soil formation, management and nutrient cycling.[5–7]

In Cuba the production of previously selected AMF for their use as biofertilizers began in the decade of 1990 with

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Table 1 – Chemical characterization of the soil collected in *Sierra de Moa*, Holguín, Cuba for the conduction of the experiment.

pH	N (%)	MO (%)	K ($mg\,kg^{-1}$)	P ($mg\,kg^{-1}$)	Ca ($mg\,kg^{-1}$)	Mg ($mg\,kg^{-1}$)	Mg:Ca ratio
6.1	0.10	2.04	51.57	<10.0	138.30	1894.53	13.70

good results in different agricultural crops.[8–10] However, the use of AMF for the restoration of degraded forest ecosystems has received poor attention, requiring a different approach. Because of the high demands of AMF propagules as part of the functional strategies of forests, the use of native soil as inoculum has been proposed.[11] In addition, this inoculum production strategy has been indicated in other parts of the world as an appropriate way to ensure the successful re-establishment of native plants in degraded soils.[12,13]

The Moa region (Holguín, Cuba) has one of the highest floristic diversity and endemism in Cuba.[14,15] It is considered the main center of evolution of the flora and vegetation of the northeastern mountains of the country.[16] These characteristics indicate the possibility of a high diverse AMF community in this region.

One of the main conservational problems in Moa results from the opencast exploration of mineral deposits, which ends up in the destruction and fragmentation of natural ecosystems. Loss of AM propagules is usually recorded following degradation of the plant cover, up to a level, that could further inhibit natural and/or artificial revegetation processes.[17] Taking into account all the previously cited aspects and the necessity of restoration in these areas, the eco-technology proposed by Torres-Arias et al.[18] represents a good alternative. It proposes the restoration of areas degraded by mining through the re-introduction of native AMF and plant species. This way, the present study pretends to determine the optimal conditions for AMF multiplication and propagule production using original soil from *Sierra de Moa* as fungal initial inoculum.

Materials and methods

The soil used in the experiment, classified as Red Ferritic, was collected in *Sierra de Moa*, which belongs to the Nipe-Sagua-Baracoa massif, Holguin, Cuba. The sampling was made at a depth of 0–20 cm. The chemical soil analyses were made at the Federal Biological Research Center for Agriculture and Forestry, Berlin, Germany (Table 1). A CHNS vario EL analyzer was used to determine the *N* content. For the rest of the elements the nitric acid extraction method was applied, using atomic absorption spectrometry (AAS) or inductively coupled plasma (ICP).

The AMF spore quantification in 100 g of the collected soil, which resulted in 585 spores, was made by wet sieving and decanting.[19,20] The rest of the soil was sieved (2 mm) and mixed with sterilized quartz sand, at a proportion of three parts of soil for one part of sand, giving place to the substrate used in the experiment.

Three plant species with different growth and development characteristics were selected for the trial: *Calophyllum antillanum* Britton, *Taliparitiaelatum* (Sw.) Fryxell and *Paspalum notatum* Flüggé. The seeds of each plant species were processed depending on their characteristics and requirements.[21] Then, they were planted in plastic pots with 1.4 kg of the previously described substrate.

The treatments included four different combinations of light (L) and temperature (T) conditions: (a) full light and high temperature (L-H) (greenhouse with natural illumination and temperature between 25 and 38 °C); (b) Shadow and high temperature (S-H) (Room temperature between 25 and 30 °C, without artificial light); (c) Artificial light and low temperature (L-C) (Air conditioned room with temperature between 19 and 23 °C); and (d) Shadow and low temperature (S-C) (Air conditioned room with temperature between 19 and 23 °C). The total duration of the experiment was of 12 months. Plants were fertilized with Long Ashton standard solution (15 mL/plant) at a 10% concentration and adjusted P content to 5 mg/L.

At the end of the experiment the following variables were measured in *C. antillanum* and *T. elatum*: height (cm), diameter at the base of the stem (cm), number of leaves, and dry weight of roots, stem and leaves independently (g). In *P. notatum*, as herbaceous plant, it was just possible to measure dry weight of leaves, stem, roots and rhizomes (g). For the three plant species an approximate amount of 0.5 g of fresh fine roots was colored to estimate the amount of external mycelium (EM) (dm^3) and percentage of colonized roots.[20,22,23]

The spore quantification in 100 g of soil from the pots was made using the previously cited methodology, followed by counting in stereoscopic microscope. AMF species identification was made based on the spores' morphology. Spores were mounted on glass slides with PVLG (Polyvinyl Lacto Glycerol) and PVLG + Melzer's reagent.

The comparisons between the treatments for each plant species were made through one-way ANOVA followed by Scott–Knott Test 5%. The statistical program used for the analyses was SISVAR 5.3.[24]

Results

Growth and development of the host plants

The three plant species showed differential growing behaviors under variable light and temperature conditions. As showed in Fig. 1, *C. antillanum* and *T. elatum* grew under the four light and temperature combinations tested. However, the biomass production of these two species varied within the treatments. On the other hand, *P. notatum* was incapable of growing under reduced light sources (shadow treatments).

The results indicate as the most proper conditions for the growing and development of *C. antillanum* those of plenty light (L-H and L-C), independently of the temperature (Table 2). Under the good illumination treatments the height and diameter were significantly superior compared to the shadow treatments (Table 2). With relation to the dry weight mean

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