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Short communication

Mycorrhizal symbiosis increase the level of total foliar phenols and tannins in *Commiphora leptophloeos* (Mart.) J.B. Gillett seedlings

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ABSTRACT

Commiphora leptophloeos (Mart.) J.B. Gillett is a Brazilian medicinal species commonly used by Brazilians for its pharmacological properties, which are related to the presence of primary and secondary plant metabolism compounds. The inoculation with arbuscular mycorrhizal fungi (AMF) in various medicinal plants increases the growth of plants and the production of therapeutically important metabolites. However, missing studies about the effects of mycorrhizal fungi on the growth and production of biomolecules in *C. leptophloeos*. The objective of this paper is to determine the growth and the concentration of primary and secondary metabolites and the total foliar antioxidant activity in *C. leptophloeos* seedlings in terms of inoculation with arbuscular mycorrhizal fungi. *C. leptophloeos* plantlets were transplanted into sacs containing 1.2 kg of soil and 10% vermicompost, which received soil-inoculum in the root region. After 140 days in a greenhouse, growth variables, primary and secondary metabolite concentration, and total foliar antioxidant activity were determined. AMF reduce the concentration of soluble carbohydrates, and increase the concentration of total foliar phenols and tannins in *C. leptophloeos* inoculated with *G. albida* and *C. etunicatum*. *A. longula* was fewer effective in relation to the other tested mycorrhizal fungi. The inoculation with *G. albida* or *C. etunicatum* favors the production of *C. leptophloeos* seedlings with increase of total foliar phenols and tannins, which are compounds with pharmacological importance.

1. Introduction

Medicinal plants are sources of phytochemicals or bioactive compounds, products which are of industrial importance in the global economy for the production of pharmaceuticals (Chaudhary et al., 2010). The use of plant species constitutes as a therapeutic resource extensively explored by the population Brazilians. The medicinal value is due the richness of active principles in the phytomass (Souza et al., 2011).

Commiphora leptophloeos (Mart.) J.B. Gillett is a Brazilian medicinal species used frequently by the population in the northeast of Brazil because of its therapeutic properties (Agra et al., 2007; Albuquerque et al., 2007; Alencar et al., 2010). This species, which is native to the semiarid region, has been used in the treatment of bronchitis, cough, renal problems, general inflammation and stomach ache (Agra et al., 2007; Albuquerque et al., 2007). The medicinal properties are related to the production of bioactive compounds such as terpenoids, triterpenes and phenolic compounds, such as tannins and flavonoids (Alencar et al., 2010). In the food and pharmaceutical industries, phenolic compounds are used as antioxidants (Rice-Evans et al., 1997). Therefore, it is necessary to use technology that favors the higher phenolic compounds production of added value.

The inoculation with arbuscular mycorrhizal fungi (AMF) in seedlings is a technology which has been tested in various medicinal plants (Ratti et al., 2010; Zubek et al., 2012), which represents a potential for the increase of plant growth (Baslam et al., 2011; Cavalcante et al.,

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2002; Manoharan et al., 2010; Santos et al., 2008; Sugai et al., 2011) and the production of therapeutically important metabolites (Karagiannidis et al., 2012; Krishna et al., 2005; Manoharan et al., 2010; Pedone-Bonfim et al., 2013; Rasouli-Sadaghiani et al., 2010; Ratti et al., 2010). However, there are few studies developed about the effects of AMF on plants native to the *Caatinga* biome.

The objective of this paper is to determine the growth and the concentration of primary and secondary metabolites and total foliar antioxidant activity in *C. leptophloeos* seedlings in terms of inoculation with arbuscular mycorrhizal fungi (AMF) to test the hypothesis that mycorrhization alters the growth and the levels of primary and secondary foliar metabolites in *C. leptophloeos* seedlings where the advantages depend on the AMF species that was used.

2. Material and methods

2.1. Substrate

The experiment was conducted in a greenhouse at the University of Pernambuco (UPE) *Campus* Petrolina, using a Latosoil (non-sterilized), which was collected in an area of the native *Caatinga* (savanna-like vegetation in Petrolina, Brazil) and used as substrate for the cultivation of the seedlings. Vermicompost was added to the soil in a proportion of 10 plus vermicompost (100 g vermicompost kg^{-1} soil). A sample of the mix was analyzed, which showed the following chemical characteristics: pH, 5.20 (H_2O – 1:2.5); organic matter, 3.21 g kg^{-1}; electric conductivity, 3.53 dS m^{-1}; P, 12.68 mg dm^{-3}; Al, 0.05 $cmol_c$ dm^{-3}; Na, 0.49 $cmol_c$ dm^{-3}; Ca, 2.70 $cmol_c$ dm^{-3}; Mg, 1.80 $cmol_c$ dm^{-3}; K, 0.26 $cmol_c$ dm^{-3} (EMBRAPA, 2011). The following native AMF species were identified in the soil: *Scutellospora* sp1, *Glomus macrocarpum* Tul. & Tul, *Apendicispora appendicula* (Spain, Sieverd. & N.C. Schenck), *Acaulospora* sp1, *Glomus* sp1 e *Acaulospora scrobiculata* Trappe. The isolates were identified considered the morphology of the glomerospores, on the basis of the Manual for Identification of AMF (Schenck and Pérez, 1990), data from International Culture Collection of Arbuscular Mycorrhizal Fungi.

2.2. Reagents

The following reagents were used: the Folin-Ciocalteau (Merck®) reagent; sodium carbonate, methyl alcohol, ethyl alcohol, sulfuric acid and glacial acetic acid (F. Maia Ltda.); phosphoric acid, pyridine, aluminum chloride, phenol, Comassie blue G-250, glucose, tannic acid and casein (Vetec Ltda.); Bovine Serum Albumin – BSA, rutin and radical DPPH (2.2-Diphenyl-1-picrylhydrazyl) (Sigma-Aldrich®).

2.3. Plant material

C. leptophloeos seeds, obtained in an area of the *Caatinga* in Petrolina, Brazil, were disinfected with sodium hypochlorite NaClO (20 mL L^{-1}) for 2 min, washed with distilled water, and germinated in trays containing soil that had previously been sterilized in an autoclave (121 °C for 30 min for 2 consecutive days).

2.4. Arbuscular mycorrhizal fungi

Cultivated AMF were granted by the Federal University of Pernambuco (UFPE), Brazil.

The AMF isolates that were used in the experiment were: *Gigaspora albida* N.C. Schenck & G.S. Sm. (UFPE 01), *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schussler (UFPE 06) e *Acaulospora longula* Spain & N.C. Schenck (UFPE 21), originating from multiplication pots in sterilized soil and organic compound (900 mL L^{-1}) in association with *Panicum miliaceum* L (Silva, 2006). The multiplied AMF were given by the Federal University of Pernambuco (UFPE), Brazil.

2.5. Mycorrhizal inoculation

C. leptophloeos plantlets with one pair of definite leaves (non-cotyledonary) were transplanted to bags with a capacity of 1.2 kg of substrate (1080 g soil plus 120 g vermicompost) and received soil-inoculum at the root region that consisting of 200 spores, hyphae, and roots colonized from either AMF isolates used: *G. albida*, *A. longula*, and *C. etunicatum*. The control did not receive soil-inoculum. Seedlings were kept in a greenhouse for 140 days, under ambient light conditions, with minimum and maximum temperatures of 18 °C and 30 °C, respectively, and a minimum and maximum relative air humidity of 34.14% and 84.23%, respectively.

2.6. Experimental setup

The experimental design was randomized with four inoculation treatments: 1) Non-inoculated control; 2) Inoculated with *Acaulospora longula*; 3) Inoculated with *Gigaspora albida*; 4) Inoculated with *Claroideoglomus etunicatum*, and with five repetitions, totaling 20 experimental units.

2.7. Plant growth parameter and AMF analysis

2.7.1. Plant growth

140 days after transplantation, the following parameters were evaluated: plant height, number of leaves, stem diameter, fresh and dry matter of the aerial and subterranean plant parts, and mycorrhizal colonization. To determine dry matter of the aerial and radicular part, the fresh matter was put in an air circulation chamber (BIOPAR, Brazil) at 45 °C until it reached constant weight.

2.7.2. AMF evaluations

To determine the mycorrhizal colonization, roots were removed from the soil, washed, and diaphanized with KOH (100 g L^{-1}) and H_2O_2 (333 mL L^{-1}), acidified with HCl (27 mL L^{-1}), and stained with Trypan blue in lactoglycerol (0.05 g L^{-1}) (Phillips and Hayman, 1970). The colonization was evaluated by means of the intersection of quadrants method (Giovannetti and Mosse, 1980).

2.8. Biochemicals and phytochemicals analysis

2.8.1. Preparation of the plant extract

500 mg of the dried leaves were punctured and transferred to amber flasks with a capacity of 80 mL to which 20 mL ethanol (950 mL L^{-1}) was added. After maceration for 12 days at 25 °C, the extract was filtered with gauze, refiltered with qualitative filter paper, and stored in amber flasks (20 mL) at − 4 °C (Brito et al., 2008).

2.8.2. Primary metabolites

2.8.2.1. Analysis of soluble carbohydrates and total proteins. Soluble carbohydrates were determined by absorbance reading in a Spectrum spectrophotometer (Shanghai, China) at 490 nm. The mixture that was analyzed consisted of an addition, in test tubes, of 50 µL of extracts, 95 µL of distilled water and 50 µL of phenol (800 g L^{-1}), and was homogenized in a Vortex Vision (Korea) shaker. Subsequently, 2 mL of sulfuric acid was added, which was quantified after 10 min in rest. Glucose was used for the standard curve (Dubois et al., 1956).

Total proteins were determined by measuring the absorbance in a spectrophotometer set at 595 nm. The analyte mixture consisted of 50 µL of extract and 2.5 mL of the Bradford reagent placed in test tubes and homogenized in a Vortex shaker and after allowing to stand for 5 min, the analyte was read in the spectrophotomete. Bovine Serum Albumin was used for the preparation of the standard curve (Bradford, 1976).