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Short communication

Arbuscular mycorrhizal fungi (AMF) affects biomolecules content in *Myracrodruon urundeuva* seedlings

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ABSTRACT

The arbuscular mycorrhizal fungus (AMF) promotes the plant growth and can alter the production of primary and secondary metabolites. *Myracrodruon urundeuva* (Engler Fr. Allemao) is an important native species that belongs to *Caatinga* biome, widely used in folk medicine in Northeast of Brazil. This species has some biological activities related to the presence of secondary metabolites, especially phenols. The aim of this work was to determine the effects of AMF on the content and concentrations of total phenols, flavonoids, soluble carbohydrate and proteins in *M. urundeuva* seedlings. Seedlings inoculated with *Acaulospora longula* (Spain & Shenck) had a higher content of soluble carbohydrate (112.7%), protein (32.87%), total phenols (81.03%) and flavonoids (57.5%) over uninoculated control. The *M. urundeuva* and *A. longula* symbiotic association promotes changes in the primary and secondary metabolism, resulting in herbage with better quality.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form a mutualistic symbiotic interaction with most of the terrestrial plants, increasing the nutrient uptake, while the host translocates 10–20% of carbohydrates from photosynthesis to fungus (Finlay, 2008; Smith and Read, 2008). These fungi are beneficial to several important plants (Rocha et al., 2006; Silva et al., 2008; Tristão et al., 2006) including the medicinal plants (Khaosaad et al., 2008; Santana, 2010). On the other hand, the AMF can alter the phytochemical synthesis both in shoots and roots (Araim et al., 2009; Baslam et al., 2011; Kapoor et al., 2004; Liu et al., 2007). This aspect of mycorrhizal symbiosis is especially important, considering that mycorrhized plants have herbage with high concentrations of bioactive molecules, making it more attractive to the pharmaceutical industry.

In this context, we have selected *Myracrodruon urundeuva* Allemão to test the effect of AMF inoculation on biosynthesis of certain biomolecules. This plant belongs to *Caatinga* ecosystem (savanna-like vegetation), and is widely used in the northeast of Brazil against genitourinary diseases, ulcers and inflammatory disturbances (Agra et al., 2007). Health professionals utilize some phytotherapics made from this species in Brazilian Public Health System (Silva et al., 2006). *Myracrodruon urundeuva* has anti-inflammatory, analgesic, bactericide and neuroprotective effects (Nobre-Junior et al., 2009; Sá et al., 2009; Viana et al., 2003). These pharmacological properties are probably due to the presence of phenolic compounds in plant extracts (Alencar et al., 2010; Araújo et al., 2008). However, despite of economic value of this species, all raw materials is obtained in an extractive way from natural plant populations. Thus, any practice employed to the rational and sustainable use can be useful for conservation of this species.

M. urundeuva produces higher concentrations of phenolic substances (Alencar et al., 2010). Phenolic compounds are biomolecules from the plant's secondary metabolism and represent some substances like tannins and flavonoids (Santos, 2007) and these molecules have pharmacological activity (Gutierrez-Lugo et al., 2004; Machado et al., 2002; Pessuto et al., 2009; Taleb-Contini et al., 2003). Therefore, it is important to choose biotechnological tools that increase the citoplasmatic concentrations of phenolics

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molecules, considering the significant pharmacological activities of *M. urundeuva*. Thereby, we choose phenolic compounds as phytochemical markers to test the influence of the AMF on this plant.

Thus, *M. urundeuva* is an excellent model to test the benefits of AMF inoculation on biosynthesis of secondary metabolites, seeing that these fungi promote *M. urundeuva* growth (Santana, 2010). Therefore, the aim of this work was to verify the influence of AMF inoculation on the total phenol, flavonoids, proteins and soluble carbohydrates content in *Myracrodruon urundeuva* seedlings. We tested the hypothesis that the mycorrhization alter the content of bioactive molecules, but the results vary according to the AMF isolate.

2. Materials and methods

2.1. Plant material, experimental setup and design

Myracrodruon urundeuva seeds were collected in the *Caatinga* area (municipality of Petrolina, NE Brazil) and the surface was sterilized by immersion for 5 min in sodium hypochlorite solution (20 mL L^{-1}). Seeds were germinated in plastic recipients with sterilized native soil (Bromex®, 980 mL of methyl bromide and 20 mL chloropicrine). Seedlings with two definite leaves were transplanted to bags with 1.2 kg of capacity containing soil plus vermicompost (100 g kg^{-1} soil).

The chemical characterization of substrate was: P – 20 mg dm^{-3}, K – 0.54 $cmol_c$ dm^{-3}, Ca – 2.0 $cmol_c$ dm^{-3}, Al – 0.05 $cmol_c$ dm^{-3}, Na – 0.13 $cmol_c$ dm^{-3}, Mg – 1.40 $cmol_c$ dm^{-3}, organic matter – 7.55 g kg^{-1}, pH – 5.80 (1:2.5, v/v) and electrical conductivity – 1.70 dS m^{-1}. The seedlings were inoculated with soil-inoculum (200 spores + colonized roots + mycelia) of the following AMF: *Acaulospora longula* Spain & N.C. Schenck and *Gigaspora albida* N.C. Schenck & G.S. Sm., multiplied in soil plus vermicompost (100 g kg^{-1} soil) (Silva, 2006), with *Panicum miliaceum* L. as host. The inoculum was stored at 4 °C (Kim et al., 2002).

The experimental design was completely randomized with three inoculation treatments: inoculated with *Acaulospora longula*, inoculated with *Gigaspora albida* and non-inoculated control in seven repetitions. The experiment was maintained in a greenhouse for 150 days under environmental conditions, with an average temperature of 25 °C, relative moisture of 77.5% and average global solar radiation of 344.2 Ly day^{-1}.

2.2. Reagents

The following reagents were used: Folin-Ciocalteau's reagent (Merk®), sodium carbonate (F.Maia Ltda.), phosphoric acid (Vetec Ltda.), methyl alcohol (F.Maia Ltda.), ethyl alcohol (F.Maia Ltda.), pyridine (Vetec Ltda.), glacial acetic acid (F.Maia Ltda.), phenol (Vetec Ltda.), aluminum chlorate (Vetec Ltda.), Comassie blue G-250 (Vetec Ltda.). For the standard curves of total proteins, soluble carbohydrate, total phenol and flavonoids we have used, respectively: BSA – Bovine serum albumin (Sigma–Aldrich®), glucose (Vetec Ltda.), tannic acid (Vetec Ltda.) and rutine hydrate (Sigma–Aldrich®).

2.3. Biochemicals and phythochemicals analysis

At the end of the experiment the shoots were collected and dried at 45 °C. Leaves were cut and samples of 500 mg were macerated in 20 mL of ethanol (950 mL L^{-1}) at 20 °C for 12 days (Brito et al., 2008). The foliar content and concentration of soluble carbohydrate, proteins, total phenols and flavonoids from ethanolic extract were quantified. The total phenol concentration, in the ethanolic extract, was determined spectrophotometrically (760 nm) by the adding of 2 mL of extract, 5 mL of Folin-Ciocalteu's phenol reagent (100 mL L^{-1}) and 10 mL of sodium carbonate (75 g L^{-1}). The volume was completed in flasks with distilled water (100 mL of capacity) and kept in the dark for 30 min (Monteiro et al., 2006). The concentration of flavonoids was determined by the adding of 1 mL of ethanolic extract, 0.6 mL of glacial acetic acid, 10 mL of pyridine-methanol (200 mL L^{-1}) and 2.5 mL of aluminum chlorate (50 g L^{-1} in methanol). The volume was completed in flasks (25 mL of capacity) and kept in the dark for 30 min and later determined spectrophotometrically (420 nm) as cited by Araújo et al. (2008). The soluble carbohydrates were determined as described by Dubois et al. (1956), by adding 50 μL of ethanolic extract, 50 μL of phenol (800 g L^{-1}), 95 μL of distilled water, 2 mL of sulfuric acid. After, the solution was stirred at vortex and kept in the dark for 10 min and then determined spectrophotometrically (490 nm). Proteins were quantified by adding 50 μL of ethanolic extract, 2.5 mL of Bradford's reagent, and then determine spectrophotometrically (595 nm) (Bradford, 1976).

2.4. Mycorrhizal colonization

Thin roots were collected and washed with tap water, clarified (KOH – 100 g L^{-1}), stained with Chlorazol black E (0.3 g L^{-1}) over night (Brundrett et al., 1984) and the mycorrhizal colonization was estimated by using the gridline intersect method (Giovannetti and Mosse, 1980).

3. Statistical analysis

Data were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey test ($p < 0.05$) using the Statistica 6.0 software (Statsoft, 2002).

4. Results and discussion

Myracrodruon urundeuva seedlings associated with *Acaulospora longula* produced more proteins, soluble carbohydrates, total phenols and flavonoids in comparison with those associated with *Gigaspora albida* or non-inoculated control (Tables 1 and 2). The mycorrhization did not affect the concentration of soluble carbohydrate, proteins, total phenols and flavonoids per gram of plant (Tables 1 and 2). Seedlings associated with *Acaulospora longula* produced more mycorrhizal structures in cortex than those associated with *Gigaspora albida* (Table 2).

The higher colonization of the seedlings inoculated with *Acaulospora longula* in comparison with those associated with *Gigaspora albida* (Table 2) reinforces the hypothesis of greater functional compatibility between symbionts (Silva, 2006), evidenced by the phytochemical data (Tables 1 and 2).

Previous studies (Santana, 2010) have shown the benefits of *Acaulospora longula* to *Myracrodruon urundeuva* growth (higher fresh and dry matter, height and number of leaves compared with seedlings associated with *G. albida* or uninoculated control) and in our work, the benefits were observed in the plants' primary and secondary metabolism (Table 2). This can be due to the higher nutrient uptake caused by the AMF colonization in the root system (Finlay, 2008; Smith and Read, 2008), resulting in major accumulation of proteins and soluble carbohydrates, considering that the nutrient content determines the maximization on the synthesis of these compounds (Wang et al., 2010). Singh et al. (2010) reported increases of 100% and 16% on production of proteins and soluble carbohydrates, respectively, in *Camellia sinensis* seedlings, with the results related to fungal inoculum used, reinforcing our initial hypothesis.

Diversely, previous studies reported that the AMF also cannot increase the content of bioactive molecules (Khaosaad et al.,