

Mycorrhizal Fungi (AMF) increase the content of biomolecules in leaves of *Inga vera* Willd. seedlings

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Received: 8 February 2015 / Accepted: 15 May 2015 / Published online: 23 May 2015
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Abstract Ingazeira (*Inga vera* Willd.), a plant native to Brazil is commonly used by Brazilians for its medicinal properties and the value of its wood. Various plants with therapeutic properties and economic importance benefit from mycorrhizal inoculation, which produces larger quantities of therapeutic compounds. However, the effects of mycorrhizal inoculation on ingazeira have not yet been studied. The objective of this paper is to evaluate the effectiveness of arbuscular mycorrhizal fungi (AMF) on the growth of seedlings and production of primary and secondary metabolites, and to determine the total foliar antioxidant activity in ingazeira seedlings. Soil-inoculum was applied to the root region of ingazeira plantlets, which were transplanted into sacs containing 1.2 kg of soil and 10 % vermicompost (100 g vermicompost kg⁻¹ soil). The inoculum consisted of 200 glomerospores per pot of each AMF: *Gigaspora albida* N.C. Schenck & G.S. Sm. (UFPE 01), *Acaulospora longula* Spain & N.C. Schenck (UFPE 21), or *Claroideoglossum etunicatum* (W. N. Becker & Gerd.) C. Walker & A. Schussler (UFPE 06). After 140 days in a greenhouse, growth variables, primary and secondary metabolite content, and total foliar antioxidant activity were determined. AMF optimized the growth and production of secondary metabolites. Mycorrhizal symbiosis can maximize growth and phytochemical production in ingazeira seedlings, thus

providing an alternative to the installation of sustainable crops of this leguminous plant.

Keywords Fabaceae · Bioactive compounds · Glomeromycota · Plant growth

1 Introduction

Inga vera Willd., which belongs to the Fabaceae family, is a fruiting and medicinal plant (Siqueira-Filho et al. 2009) often found at the margins of the São Francisco river in northeast Brazil. The nutritional value of this plant, as well as its use in apiculture and as combustible fuel is well-known (Rondon-Neto et al. 2010; Ubessi-Macarini et al. 2011); therapeutic cataplasms or compresses from the stem bark are used as astringents (Rodrigues and Carvalho 2001). In the research on foliar extracts from *I. vera*, antioxidant activity was detected (Vivot et al. 2001). This property was related to the production of bioactive substances, especially phenolic compounds, such as ellagic acid, gallic acid, and tannins (Vivot et al. 2001).

Studies on *I. vera* have demonstrated its importance for the economic development of the Brazilian semi-arid region because the species is being used for multiple purposes, especially in the wood and phytotherapeutic industries (Rodrigues and Carvalho 2001; Rondon-Neto et al. 2010; Ubessi-Macarini et al. 2011). However, overharvesting has contributed to the disappearance of the species (Silva et al. 2012). Therefore, it is necessary to use technology that favors the production of seedlings through the installation of plantations and thereby reduce the pressure of local extractivism.

The application of arbuscular mycorrhizal fungi (AMF) favors plant growth (Cavalcante et al. 2002) and results in plants with a higher concentration of therapeutically important

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compounds (Manoharan et al. 2010; Ratti et al. 2010; Wu et al. 2011; Zubek et al. 2012). These benefits have been primarily attributed to improved absorption of water and nutrients, which is the result of an extensive mycelial network that increases the radicular absorption zone and volume of explored soil (Smith and Read 2008).

Various medicinal plants benefit from inoculation with AMF, which increases the production of phytochemicals (Krishna et al. 2005; Manoharan et al. 2010; Ratti et al. 2010), and includes species from the *Caatinga* (Oliveira et al. 2013; Pedone-Bonfim et al. 2013). The symbiosis formed by such microorganisms also optimizes the growth of seedlings of native arboreal plants from the *Caatinga* biome, such as *Prosopis juliflora* (sw) DC (Aguir et al. 2004), *Anadenanthera macrocarpa* (Benth.) Brenan (Santos et al. 2008; Sugai et al. 2011), and *Anadenanthera colubrina* (vell.) Brenan (Pedone-Bonfim et al. 2013). However, there is no data available about the effects of AMF on *I. vera*.

The objectives of this study are to determine the effect of AMF inoculation on the growth of seedlings and production of primary and secondary metabolites, measure the total foliar antioxidant activity of *I. vera* seedlings after mycorrhizal inoculation, and test the hypothesis that the benefits depends on the type of AMF isolate used for inoculation.

2 Materials and methods

The experiment was carried out in a greenhouse at the University of Pernambuco (UPE) *Campus* Petrolina. Native soil (Latosoil) was used as a substrate for cultivation, which was collected in an area of the local *Caatinga* (savanna-like vegetation in Petrolina-PE, Brazil). 10 % vermicompost (100 g vermicompost kg⁻¹ soil) was added to the soil. Analysis of mix, yielded the following results: pH, 5.20 (H₂O – 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).

2.1 Reagents and standards

The following reagents were used: the Folin-Ciocalteu reagent (Merck®); sodium carbonate, methyl alcohol (99.8 % purity and 790 g L⁻¹ concentration), ethyl alcohol (99.3 % purity and 787 g L⁻¹ concentration), sulfuric acid (98 % purity and 1840 g L⁻¹ concentration), and glacial acetic acid (F. Maia Ltda.); phosphoric acid (85 % purity and 1700 g L⁻¹ concentration), pyridine, aluminum chloride, phenol (99 % purity and 1071 g L⁻¹ concentration), Coomassie blue G-250, glucose, tannic acid, and casein (Vetec Ltda.); Bovine Serum Albumin (BSA), rutin, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich®).

2.2 Plant material

Seeds from *I. vera* were obtained in an area of the *Caatinga* in Petrolina, Brazil and 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). The seedlings were considered ready for transplantation when one pair of definite leaves (non-cotyledonary) was observed.

2.3 Arbuscular mycorrhizal fungi

The following AMF isolates were used in the experiment: *Gigaspora albida* N.C. Schenck & G.S. Sm. (UFPE 01), *Claroideoglomus etunicatum* (W. N. Becker & Gerd.) C. Walker & A. Schussler (UFPE 06), and *Acaulospora longula* Spain & N.C. Schenck (UFPE 21). The isolates were harvested from multiplication vases in sterilized soil and organic compost (900 mL L⁻¹) associated with *Panicum miliaceum* L. (Silva 2006). The cultivated AMF were granted by the Universidade Federal de Pernambuco, Brazil.

2.4 Mycorrhizal inoculation and plant growth conditions

I. vera plantlets were transplanted to bags with a capacity of 1.2 kg of substrate (1080 g soil plus 120 g vermicompost) and received soil-inoculum that consisted of 200 spores, hyphae, and colonized roots from either of the three AMF isolates: *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.

2.5 Experimental design

The experimental design was randomized with four inoculation treatments and five repetitions. The treatments included the following: 1) Non-inoculated control; 2) Inoculated with *A. longula*; 3) Inoculated with *G. albida*; 4) Inoculated with *C. etunicatum*, totaling 20 experimental units.

2.6 Evaluation of plant growth and AMF analysis

140 days after transplantation, the following parameters were evaluated: plant height, number of leaves, stem diameter, levels of chlorophyll *a*, *b*, and total chlorophyll, fresh and dry matter of the aerial and subterranean plant parts, and mycorrhizal colonization.

2.6.1 Plant growth

The levels of chlorophyll *a* and, *b* and total chlorophyll were determined by using an electronic chlorophyll meter (ClorofiLOG, Falker) and the values were expressed based on the Falker Chlorophyll Index (FCI). After harvesting, the aerial part and radicular system of the plants was separated and weighed on an analytic scale (Bel Engineering, Italy) to determine fresh matter. To determine dry matter of the aerial and radicular parts, the fresh matter was placed in an air circulation oven (BIOPAR, Brazil) at 45 °C until a constant weight was obtained.

2.6.2 Evaluation of the AMF

To determine the amount of mycorrhizal colonization, roots were removed from the soil, washed, and clarified with KOH (100 g L⁻¹) and H₂O₂ (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).

2.7 Evaluation of primary and secondary metabolites

2.7.1 Preparation of plant extract

500 mg of leaves that were dried in an air circulation oven (45 °C) were punctured and transferred to amber flasks (100 mL) to which 20 mL of 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). Biomolecule contents was determined by measuring the leaf dry matter.

2.7.2 Primary metabolites

Analysis of soluble carbohydrates and total proteins Soluble carbohydrates were determined through the Dubois et al. (1956) methodology. The analyzed mixture consisted of 50 µL of extracts, 95 µL of distilled water, and 50 µL of phenol (800 g L⁻¹) placed in test tubes and homogenized in a shaker (Vortex Vision, Korea). Subsequently, 2 mL of sulfuric acid was added and after 10 min, the quantification was carried out using a spectrophotometer (Spectrum, China) at 490 nm. Glucose was used for the preparation of the standard curve.

Total proteins contents in extract samples was determined by measuring the absorbance in a spectrophotometer set at 595 nm according to the methodology proposed by Bradford (1976). The analyte mixture consisted of 50 µL of extracts and 2.5 mL of the Bradford reagent placed in test tubes and

homogenized in a Vortex shaker. 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.

2.7.3 Secondary metabolites

Analysis of total phenols, flavonoids, and tannins Total phenols were quantified by the Folin-Ciocalteu method, in samples of the prepared extract, which consisted of a mixture of 2 mL of extract, 5 mL of Folin-Ciocalteu reagent (100 mL L⁻¹), and 10 mL of sodium carbonate (75 g L⁻¹) held a volumetric flask. The volume was completed with 100 mL of distilled water and the resulting solution was stirred and allowed to stand for 30 min in the dark. After allowing to stand, readings were taken to determine absorbance in a spectrophotometer set at 760 nm. Tannic acid was used for the preparation of the standard curve (Monteiro et al. 2006).

Total flavonoids were determined with samples of the plant extract by reading absorbance levels in a spectrophotometer at 420 nm. Rutin was used for the standard curve. The analyzed mixture consisted of 1 mL of the extract, 0.6 mL of glacial acetic acid, 10 mL of 200 mL L⁻¹ pyridine solution in methanol, and a solution of 2.5 mL with 50 g L⁻¹ aluminum chloride in absolute methanol placed in volumetric flasks. The volume was completed to 25 mL with distilled water and the resulting solution was stirred, allowed to stand for 30 min, and spectrophotometric readings were taken subsequently (Araújo et al. 2008).

Total tannins were quantified by means of the methodology proposed by Monteiro et al. (2006); 6 mL of the plant extract was transferred to amber flasks, followed by the addition of 1 g of casein. The mixture was shaken (160 rpm) for 3 h at 25 °C, filtered with qualitative paper, and the resulting volume from the filtering was completed to 25 mL with distilled water. The remaining phenols were quantified by means of the Folin-Ciocalteu method. A spectrophotometric reading was taken at 760 nm, using tannic acid for the standard curve. The quantity of tannins corresponded to the difference between the values found in this final analysis and those obtained by the determination of total phenols.

2.8 Determination of the total antioxidant activity (TAA)

Total antioxidant activity was determined by means of the DPPH radical scavenging assay (2,2-Diphenyl-1-picrylhydrazyl); in the dark, 0.1 mL of the extract was transferred to test tubes with 3.9 mL of the DPPH radical (0.06 mM) and homogenized in a Vortex shaker. After allowing to stand for 30 min, the absorbance was read in a spectrophotometer (515 nm). The DPPH radical was used for the preparation of the standard curve (Rufino et al. 2007).

2.9 Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the means were compared by the Tukey test ($p < 0.05$), using the Assistat (2011) software.

3 Results and discussion

The used treatments were found to have an effect on some of the analyzed variables (Tables 1 and 2). Inoculation with AMF increased plant growth, considering the increased fresh and dry matter of the aerial part and the radicular system. *I. vera* seedlings that were inoculated with *A. longula* showed an increase of 16 % and an increase of 13 % of fresh matter (FMAP) and dry matter (DMAP) of the aerial parts, respectively, as compared to the control (Table 1). Similar results related to this growth variable have been reported in other studies (Nell et al. 2009; Samarão et al. 2011; Sugai et al. 2011; Coelho et al. 2012; Pedone-Bonfim et al. 2013). In general, mycorrhizal plants absorb more nutrients, especially those with low mobility and produce more biomass (Krishna et al. 2005; Rasouli-Sadaghiani et al. 2010; Ratti et al. 2010).

I. vera seedlings that were inoculated with *A. longula* had a higher fresh root matter (FRM) weight and dry root matter (DRM) weight by 32 % and 13 % respectively, as compared to the control (Table 1). Similar results have been reported in other studies (Toussaint et al. 2008; Samarão et al. 2011; Sugai et al. 2011; Coelho et al. 2012; Karagiannidis et al. 2012). These results are likely associated with nutritional status, which was supplied by AMF to the host plant (Santos et al. 2008; Smith and Read 2008).

With regard to plant height, stem diameter, number of leaves, and levels of chlorophyll *a* and *b* and total chlorophyll, the inoculation did not result in any benefits (Table 1). Other studies, however, have shown that mycorrhizal symbiosis promotes an increase in plant height (Santos et al. 2008; Sugai et al. 2011), stem diameter (Samarão et al. 2011; Coelho et al. 2012), number of leaves (Cavalcante et al. 2002; Aguiar et al. 2004), and levels of chlorophyll (Manoharan et al. 2010; Rahmaty and Khara 2011).

The radicular cortex of *I. vera* seedlings were colonized more by *A. longula* and *G. albida* as compared to the non-inoculated control (Table 2). The highest colonization rate was observed for *A. longula* and resulted in the highest plant growth (Tables 1 and 2), indicating that the benefit of symbiosis varies with the tested AMF, confirming the initial working hypothesis. Similar colonization results have been reported in other studies (Cavalcante et al. 2002; Karagiannidis et al. 2012).

Inoculation with AMF did not increase production of primary metabolites (Table 2). On the other hand, in other studies, mycorrhizal inoculation has been found to promote

Table 1 Plant height, number of leaves (NL), stem diameter (SD), fresh matter (FMAP), and dry matter (DMAP) of the aerial part, fresh matter (FRM) and dry matter (DRM) of the root, and levels of chlorophyll *a*, and *b* and total chlorophyll in *I. vera* seedlings, cultivated in soil and vermicompost (100 g vermicompost kg⁻¹ soil), and associated or not associated with arbuscular mycorrhizal fungi (AMF), 140 days after inoculation

Treatments	Height (cm)	NL	SD (mm)	Chlorophyll **				Total	
				FMAP (g)	DMAP (g)	FRM (g)	DRM (g)		
Control	20.40 a	9.00 a	4.06 a	8.37 b	3.12 b	8.46 b	3.43 b	12.71 a	47.98 a
<i>A. longula</i>	20.50 a	9.20 a	4.29 a	10.02 a	3.59 a	12.40 a	3.92 a	12.69 a	48.19 a
<i>G. albida</i>	20.30 a	8.20 a	4.03 a	8.67 ab	3.21 b	10.22 ab	3.69 ab	12.44 a	47.58 a
<i>C. etunicatum</i>	19.60 a	9.20 a	4.20 a	8.64 ab	3.23 b	12.24 a	3.57 ab	14.54 a	50.75 a
SD ^a	1.66	1.67	0.27	0.98	0.23	2.06	0.28	3.01	5.24

Averages ($n=5$) followed by the same letter do not differ from the Tukey test ($p < 0.05$); ^a SD standard deviation, ^b Falcker Chlorophyll Index (FCI)

Table 2 Contents of total proteins, soluble carbohydrates, total phenols, total flavonoids, total tannins, total antioxidant activity (TAA), and percentage of mycorrhizal colonization in *I. vera* seedlings, cultivated in soil and vermicompost (100 g vermicompost kg⁻¹ soil), associated or not associated with arbuscular mycorrhizal fungi (AMF), 140 days after inoculation

Treatments	Proteins (mg/plant)	Carbohydrates (mg/plant)	Phenols (mg/plant)	Flavonoids (µg/plant)	Tannins (mg/plant)	TAA(DPPH)*** (µg/plant)	Colonization (%)
Control	276.82 a	1160.52 a	6.06 b	95.48 b	5.90 b	0.24 b	31.48 b
<i>A. longula</i>	309.40 a	1517.45 a	6.96 a	123.15 a	6.75 a	0.28 a	63.08 a
<i>G. albida</i>	306.08 a	1097.98 a	6.44 ab	99.15 ab	6.26 ab	0.25 b	62.05 a
<i>C. etunicatum</i>	289.53 a	1425.23 a	6.49 ab	98.16 ab	6.34 ab	0.25 b	46.34 ab
SD*	32.65	367.31	0.52	17.74	0.50	0.01	16.67

Averages ($n=5$) followed by the same letter do not differ from the Tukey test ($p<0.05$); *SD, Standard Deviation; ***DPPH (2,2-Diphenyl-1-picrylhydrazyl).

production of carbohydrates and proteins (Thamizhiniyan et al. 2009; Ratti et al. 2010; Baslam et al. 2011; Wu et al. 2011).

The arbuscular mycorrhizal symbiosis increased the production of total phenols, flavonoids, and tannins (Table 2), however, increases in these components varied with the AMF isolate used. *I. vera* seedlings that were inoculated with *A. longula* showed an increase of 13 %, 22 %, and 13 % for total phenols, flavonoids, and tannins, respectively, as compared to the control treatment (Table 2). Similar results have been reported in other studies (Araim et al. 2009; Lee and Scagel 2009; Ceccarelli et al. 2010; Oliveira et al. 2013; Pedone-Bonfim et al. 2013).

The increase in production of secondary metabolites in the plants that formed symbiosis with *A. longula* may be related to the increased nutrient supply by AMF to the host plant (Zubek et al. 2012), which stimulated the activity of the metabolic pathways (Lohse et al. 2005; Mandal et al. 2013). The mycorrhizal colonization likely increased activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) and chalcone synthase (CHS, EC 2.3.1.74) enzymes, which are responsible for the biosynthesis of phenolic compounds (Bonanomi et al. 2001; Vermerris and Nicholson 2006).

Various mechanisms are involved with an increased production of secondary metabolites in inoculated plants (Toussaint 2007). The increased nutritional status of the inoculated plants was apparent; however, there were no differences between the levels of chlorophyll after inoculation (Table 1). Therefore, additional research is required in order to clarify the various mechanisms involved.

I. vera seedlings that were inoculated with *A. longula* showed an increase in the total foliar antioxidant activity (Table 2). The increased antioxidant activity is likely related to the increase of total levels of phenolic compounds (Degáspari and Waszczynskyj 2004), molecules that are known for their antioxidant power. In other studies, the arbuscular mycorrhizal symbiosis has also been found to

increase the total antioxidant activity (Ceccarelli et al. 2010; Hernández-Ortega et al. 2012).

The inoculated *I. vera* seedlings showed an increase in the production of phenolic compounds, which could create an additional market for this species because phenolic compounds are in high demand commercially (Degáspari and Waszczynskyj 2004) for their antioxidant, anti-inflammatory, antifungal, antiviral, and anti-allergic properties (Santos and Mello 2003; Zuanazi and Montanha 2003). The phytomass is therefore more attractive to the phytotherapeutic industry. Furthermore, the obtained benefits do not include phosphate fertilization of the cultivation substrate, which reduces the production cost of the plants. Future studies should include evaluation of the main compounds for High Performance Liquid Chromatography (HPLC) and experiments under field conditions.

4 Conclusions

1. Inoculation with *A. longula* constitutes a useful biotechnological tool for the production of *I. vera* seedlings with a large accumulation of phytomass.
2. The production of total phenols, flavonoids, tannins, and antioxidant activity is maximized by the arbuscular mycorrhizal symbiosis formed by *A. longula* in *I. vera* seedlings.
3. Mycorrhizal symbiosis can be an alternative biotechnological to increase the production of biomolecules of therapeutic importance in *I. vera* seedlings, making the phytomass more attractive to the phytotherapeutic industry.

Acknowledgments To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (processo n° 473779/2011-0) for its financial support and to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for granting a Master's scholarship to the first author and to the student Hicaro Ribeiro Soares dos Santos for his help in the installation, removal and evaluation of the experiment.

References

- Aguiar RLF, Maia LC, Salcedo IH, Sampaio EVSB (2004) Interação entre fungos micorrízicos arbusculares e fósforo no desenvolvimento da algaroba [*Prosopis juliflora* (Sw) DC]. *Rev Arvore* 28:589–598
- Araim G, Saleem A, Arnason JT, Charest C (2009) Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. *J Agric Food Chem* 57:2255–2258
- Araújo TAS, Alencar NL, Amorim ELC, Albuquerque UP (2008) A new approach to study medicinal plants with tannins and flavonoids contents from de local knowledge. *J Ethnopharmacol* 120:72–80
- Assistat (2011) [Programa de Computador]. Versão 7.6 Beta: Assistência Estatística
- Baslam M, Garmendia I, Goicoechea N (2011) Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown Lettuce. *J Agric Food Chem* 59:5504–5515
- Bonanomi A, Oetiker JH, Guggenheim R, Boller T, Wiemken A, Vögeli-Lange R (2001) Arbuscular mycorrhiza in mini-mycorrhizotrons: first contact of *Medicago truncatula* roots with *Glomus intraradices* induces chalcone synthase. *New Phytol* 150:573–582
- Bradford MM (1976) A rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Brito HO, Noronha EP, França LM, Brito LMO, Prado MS-A (2008) Análise da composição fitoquímica do extrato etanólico das folhas da *Annona squamosa* (ATA). *Rev Bras Farm* 89:180–184
- Cavalcante UMT, Maia LC, Melo AMM, Santos VF (2002) Influência da densidade de fungos micorrízicos arbusculares na produção de mudas de maracujazeiro- amarelo. *Pesq Agrop Brasileira* 37:643–649
- Ceccarelli N, Curadi M, Martelloni L, Sbrana C, Picciarelli P, Giovannetti M (2010) Mycorrhizal colonization impacts on phenolic content and antioxidant properties of artichoke leaves and flower heads two years after field transplant. *Plant Soil* 335:311–323
- Coelho IR, Cavalcante UMT, Campos MAS, Silva FSB (2012) Uso de fungos micorrízicos arbusculares (FMA) na promoção do crescimento de mudas de pinheira (*Annona squamosa* L., Annonaceae). *Acta Bot Bras* 26:933–937
- Degáspari CH, Waszczynskij N (2004) Propriedades antioxidantes de compostos fenólicos. *Visao Acad* 5:33–40
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–355
- Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA (2011) Laboratório de análises de solo e de planta. Petrolina, EMBRAPA semiárido
- Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol* 84:489–500
- Hernández-Ortega HA, Alarcón A, Ferrera-Cerrato R, Zavaleta-Mancera HA, López-Delgado HA, Mendoza-López MR (2012) Arbuscular mycorrhizal fungi on growth, nutrient status, and total antioxidant activity of *Melilotus albus* during phytoremediation of a diesel-contaminated substrate. *J Environ Manag* 95:S319–S324
- Karagiannidis N, Thomidis T, Panou-Filotheou T (2012) Effects of *Glomus lamellosum* on growth, essential oil production and nutrients uptake in selected medicinal plants. *J Agric Sci* 4:137–144
- Krishna H, Singh SK, Sharma RR, Khawale RN, Grover M, Patel VB (2005) Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization. *Sci Hortic* 106:554–567
- Lee J, Scagel CF (2009) Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chem* 115:650–656
- Lohse S, Schliemann W, Ammer C, Kopka J, Strack D, Fester T (2005) Organization and metabolism of plastids and mitochondria in arbuscular mycorrhizal roots of *Medicago truncatula*. *Plant Physiol* 139:329–340
- Mandal S, Evelin H, Giri B, Singh VP, Kapoor R (2013) Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside - A in *Stevia rebaudiana* via nutritional and non-nutritional mechanism. *Appl Soil Ecol* 72:187–194
- Manoharan PT, Shanmugaiiah V, Balasubramanian N, Gomathinayagam S, Sharma MP, Muthuchelian K (2010) Influence of AM fungi on the growth and physiological status of *Erythrina variegata* Linn. grown under different water stress conditions. *Eur J Soil Biol* 46: 151–156
- Monteiro JM, Albuquerque UP, Lins Neto EM, Araújo EL, Albuquerque MM, Amorim ELC (2006) The effects of seasonal climate changes in the Caatinga on tannin levels in *Myracrodruon urundeuva* (Engl.) Fr. All, and *Anadenanthera colubrina* (Vell.) Brenan. *Braz J Pharmacogn* 16:338–344
- Nell M, Vötsch M, Vierheilig H, Steinkellner S, Zitterl-Eglseer K, Franz C, Novak J (2009) Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). *J Sci Food Agric* 89:1090–1096
- Oliveira MS, Campos MAS, Albuquerque UP, Silva FSB (2013) Arbuscular mycorrhizal fungi (AMF) affects biomolecules content in *Myracrodruon urundeuva* seedlings. *Ind Crop Prod* 50:244–247
- Pedone-Bonfim MVL, Lins MA, Coelho IR, Santana AS, Silva FSB, Maia LC (2013) Mycorrhizal technology and phosphorus in the production of primary and secondary metabolites in cebil (*Anadenanthera colubrina* (Vell.) Brenan) seedlings. *J Sci Food Agric* 92:1479–1484
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55:158–161
- Rahmaty R, Khara J (2011) Effects of vesicular arbuscular mycorrhizal *Glomus intraradices* on photosynthetic pigments, antioxidant enzymes, lipid peroxidation, and chromium accumulation in maize plants treated with chromium. *Turk J Biol* 35:51–58
- Rasouli-Sadaghiani M, Hassani A, Barin M, Danesh YR, Sefidkon F (2010) Effects of arbuscular mycorrhizal (AM) fungi on growth, essential oil production and nutrients uptake in basil. *J Med Plant Res* 4:2222–2228
- Ratti N, Verma HN, Gautam SP (2010) Effect of *Glomus* species on physiology and biochemistry of *Catharantus roseus*. *Indian J Microbiol* 50:355–360
- Rodrigues VEG, Carvalho DA (2001) Levantamento etnobotânico de plantas medicinais no domínio cerrado na região do alto Rio Grande – Minas Gerais. *Cienc Agrotecnol* 25:2–123
- Rondon-Neto RB, Santos JS, Silva MA, Koppe VC (2010) Potencialidades de uso de espécies arbustivas e arbóreas em diferentes fisionomias de cerrado, em Lucas do Rio Verde/MT. *Rev Biol Ciênc Terra* 10:113–126
- Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J, Saura-Calixto FD (2007) Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. Comunicado Técnico 127. Fortaleza, Embrapa Agroindústria Tropical
- Samarão SS, Rodrigues LA, Martins AM, Manhães TN, Alvim LAM (2011) Desempenho de mudas de gravioleira inoculadas com fungos micorrízicos arbusculares em solo não-esterilizado, com diferentes doses de fósforo. *Acta Sci Agron* 33:81–88
- Santos SCM, Mello JCP (2003) Taninos. In: Simões CMO, Schenkel EP, Gosmann G, Pallazzo DE, Mello JC, Mentz LA, Petrovick PR (eds) *Farmacognosia: da planta ao medicamento*, 5th edn. UFRGS, Porto Alegre, pp 615–656

- Santos DR, Costa MCS, Miranda JRP, Santos RV (2008) Micorriza e rizóbio no crescimento e nutrição em N e P de mudas de angico-vermelho. *Rev Caatinga* 21:76–82
- Silva FSB (2006) Fase assimiótica, produção, infectividade e efetividade de fungos micorrízicos arbusculares (FMA) em substratos com adubos orgânicos. Tese de doutorado, Universidade Federal de Pernambuco
- Silva LF, Silva ML, Cordeiro AS (2012) Análise do mercado mundial de madeiras tropicais. *Rev Polit Agric* 21:48–54
- Siqueira-Filho JA, Santos APB, Nascimento MFS, Espírito Santo FS (2009) Guia de Campo de Árvores da Caatinga. Editora e gráfica Franciscana Ltda, Petrolina
- Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Academic Press, London
- Sugai MAA, Collier LS, Saggin-Júnior OJ (2011) Inoculação micorrízica no crescimento de mudas de angico em solo de cerrado. *Bragantia* 70:416–423
- Thamizhiniyan P, Panneerselvam M, Lenin M (2009) Studies on the growth and biochemical activity of *Coleus aromaticus* Benth. as influenced by AM fungi and *Azospirillum*. *Recent Res Sci Technol* 1:259–263
- Toussaint J-P (2007) Investigating physiological changes in the aerial parts of AM plants: what do we where should we be heading? *Mycorrhiza* 17:349–353
- Toussaint J-P, Kraml M, Nell SE, Smith FA, Steinkellner S, Schmiderer C, Vierheilig H, Novak J (2008) Effect of *Glomus mosseae* on concentration of rosmarinic and caffeic acids and essential oil compounds in basil inoculated with *Fusarium oxysporum* f. sp. *Basilica*. *Plant Pathol* 57:1109–1116
- Ubessi-Macarini C, Negrelle RRB, Souza MC (2011) Produtos florestais não-madeiráveis e respectivo potencial de exploração sustentável, associados à remanescente florestal ripário do alto rio Paraná, Brasil. *Acta Sci Biol Sci* 33:451–462
- Vermerris W, Nicholson R (2006) Phenolic compound biochemistry, p. 267
- Vivot E, Munoz JD, Cruanes MC, Cruanes MJ, Tapia A, Hirschmann GS, Martínez E, Di Sapio O, Gattuso M, Zacchino S (2001) Inhibitory activity of xanthine-oxidase and superoxide scavenger properties of *Inga verna* subsp. *affinis*. Its morphological and micrographic characteristics. *J Ethnopharmacol* 76:65–71
- Wu Q-S, Zou Y-N, He X-H, Luo P (2011) Arbuscular mycorrhizal fungi can alter some root characters and physiological status in Trifoliate orange (*Poncirus trifoliata* L. Raf.) seedlings. *Plant Growth Regul* 65:1–3
- Zuanazi JAS, Montanha JA (2003) Flavonóides. In: Simões CMO. In: Schenkel EP, Gosmann G, Pallazzo DE, Mello JC, Mentz LA, Petrovick PR (eds) *Farmacognosia: da planta ao medicamento*, 3rd edn. UFRGS, Porto Alegre, **cap.23**
- Zubek S, Mielcarek S, Tumau K (2012) Hypericin and pseudohypericin concentrations of a valuable medicinal plant *Hypericum perforatum* L. are enhanced by arbuscular mycorrhizal fungi. *Mycorrhiza* 22: 149–156