## Arbuscular mycorrhizal fungi and phosphate fertilization on star fruit tree seedlings

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## ABSTRACT

The success of star fruit cultivation deployment begins with the use of good quality seedlings Thus the inoculation of mycorrhizal fungi in culture seedling can be an alternative, since it was found success in both growth as nutritional aspect fruit species. In this sense, the aim of this study was to evaluate the effects of arbuscular mycorrhizal fungi (AMFs) on growth and mineral composition of star fruit seedlings, cultivated under different doses of phosphorus (P). The experiment was conducted in a greenhouse and the experimental design used was randomized blocks in a 3 x 4 factorial scheme, with three microbiological treatments (without inoculation, *Rhizofagus clarum, Glomus etunicatum*) and four doses of P (0, 50, 100 and 200 mg kg<sup>-1</sup> soil) with four replications. Plants were harvested four months after sowing for biometric and nutritional analysis. In the soil without phosphate fertilization, *R. clarum*, provided increments of 49% in height, 99% in dry matter production and 86, 129 and 108% in the contents of N, K, and Ca, respectively, in relation to the control. Regardless of phosphate fertilization, the content of P, Mg, and S in the dry matter weight was 19.2, 17.6 and 23.6% higher in the treatment inoculated with *R. clarum*, in relation to the control. Mycorrhizal dependence of star fruit tree varied according to fungus species and the P dose used, being greater when the fungus *R. clarum* was used in absence of P.

Key words: Averrhoa carambola L.; mineral composition; mycorrhiza

# Fungos micorrizicos arbusculares e adubação fosfatada em mudas de caramboleira

#### RESUMO

O sucesso da implantação do cultivo da caramboleira inicia-se com a utilização de mudas de boa qualidade, nessa linha a inoculação de fungos micorrizicos em mudas da cultura pode ser uma alternativa, visto que foi verificado sucesso tanto no crescimento como em aspecto nutricionais em espécies frutíferas. Nesse sentido, objetivou-se com esse trabalho avaliar a inoculação de fungos micorrízicos arbusculares (FMAs) no crescimento e composição mineral das mudas de caramboleira, cultivadas com doses de fósforo (P). O delineamento estatístico utilizado foi de blocos ao acaso, num fatorial 3 x 4, com três tratamentos microbiológicos (sem inoculação, *Rhizofagus clarum, Glomus etunicatum*) e quatro doses de P (0, 50, 100 e 200 mg kg<sup>-1</sup> de solo) e quatro repetições. As plantas foram colhidas aos quatro meses após a semeadura para análises biométricas e nutricionais. No solo sem adubação fosfatada, o fungo *R. clarum* proporcionou incrementos de 49% na altura, 99% na produção de massa de matéria seca e de 86, 129 e 108% nos conteúdos de N, K e Ca, respectivamente, em relação ao controle. Independente da adubação fosfatada, o conteúdo de P, Mg e S na massa de matéria seca foi 19,2, 17,6 e 23,6% maior no tratamento inoculado com *R. clarum*, em relação ao controle. A dependência micorrízica da caramboleira variou de acordo com a espécie de fungo e a dose de P utilizada, sendo maior quando se utilizou o fungo *R. clarum* na ausência de P.

Palavras-chave: Averrhoa carambola L.; composição mineral; micorriza

#### Introduction

Star fruit (*Averrhoa carambola* L.), a species originated in Asia and in the family Oxalidaceae, has a great potential to be better exploited in Brazil, mainly for being adapted to tropical climates, without occurrence of frosts, and for its great acceptance by Brazilian and global consumers in the market of exotic fruits (Bastos, 2004).

Although it presents a productive potential, which can reach 60 t ha<sup>-1</sup> (Bastos, 2004), star fruit tree is still grown under low technological conditions, mainly due to the lack of cultivation techniques and/or varieties adapted to the different growing conditions. Most of the orchards in production in Brazil were formed from seedlings derived from cultivars in Florida and Malaysia (Bastos et al., 2009).

The beneficial effects of arbuscular mycorrhizal fungi have been demonstrated in the most varied conditions and plant species, in most cases, stimulating plant growth as a result of its effect on plant nutrition, especially in increasing the absorption of phosphorus (Freitas et al., 2004; Santos et al., 2011; Heitor et al., 2016; Pereira et al., 2016).

The production of star fruit seedlings is one of the limiting factors to the commercial expansion of the culture, due to the time it takes to be formed and begin to set fruit. An alternative to the preparation of these seedlings is to use AMFs, which have contributed to reduce the production time of several fruit trees such as citrus (Altoé et al., 2008), yellow passion fruit (Cavalcante et al., 2001; Cavalcante et al., 2002), sweet passion fruit (Anjos et al., 2005; Vitorazi et al., 2012), papaya (Lima et al., 2011) and cashew (Weber et al., 2004), in addition to reducing phosphate fertilization (Riter Netto et al., 2014).

Considering that there are few studies on star fruit crops in Brazil and that there are no reports on the relation between mycorrhizal symbiosis and seedling production of this fruit tree, the present study aimed to evaluate the effects of mycorrhizal fungi and phosphate fertilization on growth and mineral composition of star fruit seedlings.

#### **Materials and Methods**

The experiment was conducted in a greenhouse at the Universidade Estadual do Norte Fluminense, located in Campos dos Goytacazes, RJ (21° 19' 2" S; 41° 10' 40" W; at an elevation of 14 m above sea level). During the experiment period, daily minimum temperature ranged from 12.5 °C to 24.0 °C, the average temperature was 19.9 °C and the maximum daily temperature ranged from 21°C to 40°C with an average of 32.9 °C. The experimental design was randomized blocks in a 3x4 factorial combination of three microbiological treatments (without inoculation, *Rhizophagus clarum, Glomus etunicatum*) and four doses of phosphorus (0, 50, 100 and 200 mg kg<sup>-1</sup> soil), with four replications. The experimental unit was composed of a plastic pot containing 3 kg of soil and five plants per vase.

The substrate used for the experiment was soil collected at 0-20 cm depth, sieved, mixed with sand 1:2 (v/v) and sterilized in an autoclave twice at a temperature of 121°C for 1 hour. After autoclaving, the substrate presented the following chemical characteristics: pH in water = 5.5; organic matter = 13.79 g dm<sup>-3</sup>; P = 4.0 mg dm<sup>-3</sup>; S = 19.0 mg dm<sup>-3</sup>; K<sup>+</sup>= 1.6 mmol<sub>c</sub> dm<sup>-3</sup>; Ca<sup>2+</sup> = 8.0 mmol<sub>c</sub> dm<sup>-3</sup>; Mg<sup>2+</sup>= 6.4 mmol<sub>c</sub> dm<sup>-3</sup>; Al<sup>3+</sup>= 1.0 mmol<sub>c</sub> dm<sup>-3</sup>; H+Al= 15.3 mmol<sub>c</sub> dm<sup>-3</sup>; SB = 16.40 mmol<sub>c</sub> dm<sup>-3</sup>; T = 31, 70 mmol<sub>c</sub> dm-3; Fe = 56.17 mg dm<sup>-3</sup>; Cu = 0.15 mg dm<sup>-3</sup>; Zn = 1.79 mg dm<sup>-3</sup>; Mn = 25.25 mg dm<sup>-3</sup> and B = 0.45 mg dm<sup>-3</sup>.

A dose of 20 mg kg<sup>-1</sup> of N as  $NH_4NO_3$  was applied for each treatment. 120 mg kg<sup>-1</sup> of KCl was used in treatment 0 P for K-correction. For treatment 50 P, 219.5 mg kg<sup>-1</sup> of  $KH_2PO_4$  was used for the correction of K and P. For treatment 100 P, we used 219.5 mg kg<sup>-1</sup> of  $KH_2PO_4$  and 222.5 mg kg<sup>-1</sup> of  $NaH_2PO_4$   $H_2O$ . Finally, 219.5 mg kg<sup>-1</sup> of  $KH_2PO_4$  and 667.3 mg kg<sup>-1</sup> of  $NaH_2PO_4$   $H_2O$  were used in 200 P dose. After fertilization, the soil was incubated for 15 days, and kept properly moistened.

The substrate used for inoculum multiplication was a mixture of soil and sand 1:2 (v/v), sterilized in an autoclave twice, at 121 °C, for 1 hour. 50 g of initial inoculum were added to the substrate, placed in 3kg pots, constituting a mixture of soil containing spores, hyphae and colonized roots, with the fungus of each species to be studied: *R. clarum* and *G. etunicatum*. The initial inoculum of the fungus was removed from the collection of fungal species of the Soil Microbiology Sector of UENF. Then, 15 seeds of *Brachiaria brizantha*, disinfested in a 0.5 % sodium hypochlorite solution for 10 minutes and rinsed with deionized water, were sown in each pot. Four months after sowing, shoot was cut and, thirty days after cutting, the soil mixture containing spores, hyphae and sliced thin roots was used as inoculum.

Star fruit seeds cv. *Malasia* were placed in water for four hours before planting. Ten seeds were planted per pot. After germination and seedling development has begun, a thinning was carried out, leaving five plants per pot. Before sowing, 50 g of AMFs species inoculum were added to the inoculated treatments. Inoculants were placed 2-3 cm below the soil surface. The plants were irrigated daily, using deionized water.

The plants were harvested four months after planting. Average height and shoot dry mass were determined after drying in an oven of forced air ventilation at a temperature of 65 °C for 48 hours. After being dried, the samples were ground in a Willey Mill and stored in hermetically sealed bottle (Malavolta, 1997). Fine roots were collected, washed with water, cut approximately 2 inches in length and stored in 50 % ethanol for further assessment of root colonization percentage. Roots were colored according to (Grace & Stribley, 1991), with adaptations, to determine the percentage of mycorrhizal colonization. The roots were taken under a microscope in order to observe the presence of structures of AMFs.

Levels of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) in the shoot were determined. Plant material was subjected to oxidation by sulfuric digestion and the extract was obtained, from which nitrogen was determined by Nessler method (Jackson, 1965), phosphorus by molybdate colorimetric method (Malavolta, 1997) and potassium by flame emission spectrophotometry. Ca, Mg and S were quantified, after oxidation of plant material by digestion nitro-percloric, by atomic absorption spectrometry and by turbidimetry with barium chloride.

Relative mycorrhizal dependence (RMD) of star fruit trees was assessed through the ratio between the increase in shoot dry matter production of mycorrhizal plants compared to nonmycorrhizal ones and shoot dry matter of non-mycorrhized plants, with the result expressed as a percentage (Bethlenfalvay et al., 1982).

Analyses of variance were performed for quantitative variables, averages of microbiological treatments were compared by Tukey test at 5 % probability, and P doses were analyzed by polynomial regression.

## **Results and Discussion**

Effects of interactions between fungal species and P doses on shoot dry matter production and height of star fruit tree were observed (Table 1). In the absence of phosphate fertilization, shoot dry matter production and height of star fruit tree, in treatments inoculated with R. clarum, were 99 and 49 % higher, respectively, than the results obtained in the control treatment, showing the efficiency of this fungal species in promoting star fruit tree growth in the absence of P. The estimated doses of P of 125.40; 143.80 and 160.45 mg kg<sup>-1</sup> soil, respectively for G. etunicatum, without inoculation, and R. clarum were the ones that provided the greatest dry matter production. Results similar to those found in the present study for the species R. clarum at P dose of 0 were observed by Freitas et al. (2006) in Mentha arvensis, by Soares & Martins (2000) in Passiflora edulis, by Samarão & Martins (1999) in Psidium guajava L. and by Sato et al. (1999), with Heliconia sp. and Gerbera sp.

When phosphate fertilization was not used, the highest percentage of mycorrhizal colonization was 85%, observed in the treatment with *R. clarum* (Figure 1), which provided greater dry matter production (Table 1). The increase of phosphate fertilization resulted in reduced mycorrhizal colonization in star fruit seedlings inoculated with the fungus *R. clarum*. According to (Smith & Read, 1997), in soils with low availability of P the highest mycorrhizal colonization is usually followed by stimuli in plant growth; however this stimulus has not been observed for star fruit plants inoculated with *Glomus etunicatum*.

The observed differences in dry matter production of star fruit tree among the different microbiological treatments can, according to (Sieverding, 1991), occur because arbuscular mycorrhizal fungi demonstrate preference for specific hosts, and symbiotic efficiency is influenced by plant and fungus genotypes, as well as by environmental conditions.

According to Moreira & Siqueira (2002), the decrease in mycorrhizal colonization with increased P doses can be



Figure 1. Mycorrhizal colonization (%) of star fruit tree roots in relation to microbiological treatments and P doses

explained by phosphatase activity in roots, which is low. As a result, the lecithin found in these roots is freed and binds to carbohydrates of the mycorrhizal fungus, inhibiting its growth. On the other hand, phospholipid biosynthesis is increased and, as a result, cell permeability, root exudation of amino acids, root colonization and infection are decreased. Higher doses of P increased photosynthesis and assimilate availability on the roots by inhibiting the mycorrhizal fungus propagules. Several studies have demonstrated that different species of AMFs should be tested in a same plant under the same environmental conditions to select efficient AMFs for their ability to promote the growth of their host (Melloni et al., 2000; Bressan et al., 2001; Weber et al., 2004; Freitas et al., 2006). It should be emphasized that in the control treatment the value found for mycorrhizal colonization was zero.

For the contents of N, K, and Ca, interaction between species of fungi and P doses were observed (Figures 2, 3 and 4), respectively. In the absence of phosphate fertilization, the contents of N, K, and Ca in shoot dry matter were 86, 108 and 129%, respectively, higher than the contents found in treatments inoculated with *R. clarum* in comparison to the control treatment. Bressan et al. (2001) observed that arbuscular mycorrhizal fungi inoculation increased foliar concentrations of N and K. Melloni et al. (2000) also found interactions in the contents of N, K, and Ca of the shoot. The estimated P doses of 162.6, 206.1 and 131.9 mg kg<sup>-1</sup> soil for the control, *R. clarum* and *G. etunicatum*, respectively, were

Table 1. Shoot dry matter production and height of star fruit plants in relation to microbiological treatments and P doses

Fungus	Shoot dry matter (g pot <sup>1</sup> )					Height (cm plant <sup>-1</sup> )					
	P (mg kg <sup>-1</sup> soil)				Auguaga	P (mg kg <sup>-1</sup> soil)				Avenage	
	0	50	100	200	Average	0 50	100	200	Average		
R. clarum	4.00 a	7.67 ab	8.10 a	8.30 a	7.02	17.8 a	24.0 a	23.9 a	23.2 a	22.2	
G. etunicatum	2.29 b	8.00 a	7.84 a	6.96 b	6.27	12.4 b	23.2 a	23.1 a	22.0 a	20.2	
Without inoculation	2.01 b	6.47 b	7.36 a	7.57 ab	5.85	11.9 b	22.8 a	22.7 a	22.5 a	20.0	
Average	3.09	6.52	8.40	7.50		14.8	21.3	24.7	22.3		
CV (%)	10.88					7.99					

The averages followed by the same letter, uppercase letter in the column and lowercase letter in the line, do not differ from each other, based on the Tukey test (P<0,05)



Figure 2. N contents (mg pot<sup>1</sup>) in leaf dry matter of star fruit seedlings in relation to microbiological treatments and P doses



Figure 3. K content (mg pot<sup>1</sup>) in leaf dry matter of star fruit seedlings in relation to microbiological treatments and P doses

the ones that achieved the largest increments in N content in shoot dry matter. For K, P estimated doses were 128.3, 126.5 and 123.1 mg kg<sup>-1</sup> soil; and for Ca, P estimated doses were 117, 110.6 and 110.8 mg kg<sup>-1</sup> soil for the control, *R. clarum* and *G. etunicatum*, respectively.

The contents of P, Mg, and S in shoot were influenced by both microbiological treatments and P doses (Table 2). Regardless of phosphate fertilization, plants inoculated with *R. clarum* presented greater contents of P, Mg, and S than the ones in the control treatment, with increments of 19.2, 17.6 and 23.7 percent, respectively. According to P doses, the contents of P (P =  $-0.0005x^2 + 0.20x + 1.85$ , R<sup>2</sup> =  $0.99^{**}$ ), Mg (Mg =  $-0.0013x^2 + 0.327x + 10.52$ , R<sup>2</sup> =  $0.89^{**}$ ) and S (S =  $-0.00061x^2$ + 0.1846x + 3.83, R<sup>2</sup> =  $0.98^*$ ) showed quadratic responses. Freitas et al. (2006) working with *M. arvensis* L., found similar results for P contents.

The greatest value for mycorrhizal dependence was observed in plants inoculated with the fungus R. *clarum* and in the absence of P (Figure 5). As the doses of P were



Figure 4. Ca content (mg pot<sup>1</sup>) in leaf dry matter of star fruit seedlings in relation to microbiological treatments and P doses

Table 2. Contents of P, Mg, and S in shoot dry matter mass of star fruit tree in relation to microbiological treatments and P doses

Tuestment	Р	Mg	S
Ireatment		(mg pot <sup>-1</sup> )	
R. clarum	14.6 a	23.4 a	13.5 a
G. etunicatum	12.2 b	22.2 ab	11.2 ab
Without inoculation	11.6 b	19.9 b	10.9 b
CV (%)	14.9	14.1	23.2





P doses (mg kg<sup>-1</sup>)

Figure 5. Mycorrhizal dependency of star fruit tree in relation to microbiological treatments and P doses

incremented, mycorrhizal dependency values decreased, while in plants grown with the fungus *G. etunicatum* at 200 P dose the value was negative. This means that the fungus *G. etunicatum* provided a reduction in dry matter production compared with the control treatment at the mentioned P dose. Melloni et al. (2000) working with 'Rangpur' lime, verified a decrease in the percentage of mycorrhizal dependence with increased doses of P in the substrate, and that the fungus *G. etunicatum* presented negative mycorrhizal dependency values. Freitas et al. (2006) working with *M. arvensis* L., also found similar results.

## Conclusions

In the absence of phosphate fertilization, inoculation of star fruit seedlings with *R. clarum* promoted greater increments in dry matter production, height and in the contents of nitrogen, potassium, and calcium.

The contents of phosphorus, magnesium and sulphur were greater in star fruit seedlings inoculated with *R. clarum*, regardless of phosphate fertilization.

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