Crop Production

Interaction of papaya seedlings inoculated with different mycorrhizal fungi species[1]

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ABSTRACT

Papaya (*Carica papaya* L.) is one of the most cultivated fruits in Brazil. Thus, increasing efforts to improve the crop efficiency have been carried out, being the study of quality seedling production of fundamental importance. Arbuscular mycorrhizal fungi, in symbiosis with the root system of plants, can bring great improvements to the morphophysiological aspects of the papaya tree. The aim of this study was to evaluate the interaction of papaya seeds treated with AMF, in order to support management works on the crop. The experiment was installed in an agricultural greenhouse in a completely randomized design with three treatments: T0 – control (no inoculation); T1 - inoculation of seeds with *Gigaspora rosea* + *Gigaspora margarita*; and T2 - inoculation of seeds with *Rhizophagus clarus*, with 40 replicates, with each sowing cell being considered a replicate. Mycorrhizal colonization, seedling emergence and biometric indices at 60 days after sowing were evaluated. High symbiosis rate was observed between papaya seedlings with *Rhizophagus clarus* and *Gigaspora rosea* + *Gigaspora margarita*. Increase in the percentage and speed emergence and decrease in emergence time in relation to control was observed, in addition to increase in biometric characteristics of seedlings, evidencing its beneficial use for higher production.

Keywords: *Carica papaya* L.; arbuscular mycorrhizal fungi; symbiosis; seedling quality; sustentability.

INTRODUCTION

Papaya (*Carica papaya* L.), belonging to the Caricacea family, is one of the major fruit crops cultivated in tropical and sub-tropical zones (Sekeli *et al.*, 2018), being popularly known for its nutritional and therapeutic properties (Singh *et al.*, 2020).

Its highest production occurs in tropical and subtropical regions, with Central and South America accounting for 47% of fruit production, produced all year round, with high availability in the market (Santana *et al.*, 2019). In this scenario, Brazil occupies the third position in terms of world papaya production, whose production is over 1 million tons, which corresponds to 8.4% of the total world production, only behind India and the Dominican Republic, respectively (Serafini *et al.*, 2021).

There are three methods of propagating papaya plants, these are: through seeds, grafting and cuttings. In commercial planting in nurseries, the most widely used method is seed.

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However, papaya productivity has declined, along with challenges related diseases, which could jeopardize production (Hariono *et al.*, 2021). Strategies to control and/or manage crop diseases effectively use a combination of cultural, biological and chemical tools, but damping-off control is difficult, requiring the adoption of prophylaxis measures with the use of seed treatments and transplants before seeds or plants are placed in the field (Lin *et al.*, 2012).

In this sense, the use of arbuscular mycorrhizal fungi (AMF) can bring benefits to the production of quality papaya seedlings, since plants in symbiosis with AMFs undergo biochemical, physiological and molecular changes related to their defense system so that symbiosis is established (García-Garrido & Ocampo, 2002), improving their tolerance to biotic and abiotic factors (Hu *et al.*, 2015; Hage-Ahmed *et al.,* 2019).

Thus, inoculation with AMFs in the production phase of papaya seedlings can become an alternative technology with potential for practical application. Therefore, the present study aimed to evaluate the effect of inoculation of different arbuscular mycorrhizal fungi (AMFs) on papaya seeds, aiming at the formation of healthy seedlings in order to subsidize management and crop production studies.

MATERIAL AND METHODS

The experiment was carried out in a Pad&Fan greenhouse, located in the municipality of Dracena, São Paulo, Brazil (21º27’ S, 51º33’ W, altitude 421 m) with automatic microsprinkler irrigation with flow rate of 118 Lh^{-1}.

According to the Köppen classification, the predominant climate in the region is Aw, categorized as humid subtropical, with hot and rainy summers and dry and mild winters with low rainfall, with average annual temperature of 23.6 °C (Rodrigues *et al.*, 2020).

Commercial papaya seeds of the Formosa group “Taiuning 1” cultivar Feltrin® brand were sown in expanded polystyrene trays containing 36 cells filled with Carolina Soil® commercial agricultural substrate, which was autoclaved for 4 hours, so that no microbial interference occurred in treatments with AMF inoculation, being sown 3 seeds per cell.

For this research, the treatments consisted of the species of arbuscular mycorrhizal fungi (AMF), previously identified as *Gigaspora* rosea + *Gigaspora* margarita and *Rhizophagus* clarus belonging to the collection of the School of Agriculture “Luiz de Queiroz” (ESALQ), University of São Paulo, Soil Science Department, Soil Microbiology Laboratory. The mycorrhizae grown on soil.

The experiment was conducted in a completely randomized design containing 3 treatments, namely: T0 – no inoculation, with cells filled only with 500g of Carolina Soil® commercial agricultural substrate not autoclaved; T1 - inoculation with 20g the soil with *Gigaspora rosea* + *Gigaspora margarita* (GR+GM) added to 480g of autoclaved substrate; and T2 - inoculation with 20g the soil with of *Rhizophagus clarus* (RC) added to 480g of autoclaved substrate, with 40 replicates, with each cell considered an experimental unit. Inoculation was performed when the seeds were sown in the cells.

Five days after sowing, the following evaluations were performed until seedling emergence stabilization: emergence (%); emergence speed index and mean emergence time ($days^{-1}$). In addition, the Relative Growth Rate (RGR) was evaluated, considering the increase in seedling mass per unit of original mass, measured by its length, in centimeters, every 10 days, using the formula used by Benincasa (2003):

$RGR = (\ln R2 - \ln R1)/\Delta T$ where,

ln = neperian logarithm

R1 = initial branch length;

R2 = final branch length;

ΔT = period, in days, that delimits the beginning and end of the branch length measurement.

Sixty days after sowing, 20 seedlings were randomly sampled from each treatment for the following biometric evaluations: stem diameter (mm), using digital caliper and determined close to the plant neck; shoot length (cm), measured from the plant neck to the last leaf with graduated ruler; root length (cm), measured from the plant neck to the root region with graduated ruler; seedling fresh mass, weighing whole seedlings on scale with precision of 0.001 g (1 mg), obtaining the seedling fresh mass of each treatment, expressed in $grams.seedling^{-1}$; seedling dry mass, obtained from seedlings sampled from each treatment and replicate dried in oven regulated at 60 ºC, for 48 hours, until constant dry mass is obtained, measured on analytical scale with precision of 0.0001 g, with results expressed in g $seedling^{-1}$.

In addition, in order to verify the efficiency of seed inoculation with AMFs the inoculated plants were evaluated for the percentage of colonization by the histochemical staining method: the non-vital method of Phillips & Hayman (1970), using trypan-blue dissolved in lactoglycerol.

From results obtained, statistical analysis of all accessions was performed, grouping the averages obtained by the Tukey test at 5% probability level. To perform statistical analyses, the Sisvar software was used: a computer statistical analysis system, version 5.6 (Ferreira, 2019).

The Relative Growth Rate (RGR) was adjusted in relation to time using a second-degree polynomial equation. Therefore, data were presented in the form of graphs, evaluating the behavior of curves (Ferreira *et al.*, 2019). The percentage of colonization of the root system of papaya seedlings by mycorrhizas was also presented in the form of graph.

RESULTS AND DISCUSSION

From results presented in Figure 1, it could be observed that there was efficient inoculation of the mycorrhizal species used in the root system of papaya seedlings.

Seedlings inoculated with *Rhizophagus clarus* and *Gigaspora rosea* + *Gigaspora margarita* showed inoculation percentage of 77.8% and 75.4%, respectively, which is considered a satisfactory degree of root colonization according to the, when compared with some results found in the literature. Chatzistathis *et al.* (2013), concluded the, in generally, AMF colonization of olive plants (45-73%) was satisfactory. Chiomento *et al.* (2020), working with *Rhizophagus clarus* mycorrhizal colonization of roots of goldenberry plants, found 57% of root colonization and discuted that although mycorrhizal colonization is important, the percentage of root infectivity is not always correlated with the efficiency of symbiosis (Konvalinková & Jansa, 2016).

As indirect effects on arbuscular mycorrhizal symbiosis mediated by changes in the physiology of the host plant, modification of root morphology and increased biosynthesis of antioxidants stand out, in addition to influence on seed germination (Bennett & Meek, 2020).

Regarding germination variables presented in Table 1, it was observed that there was statistical difference for all analyzed variables.

Regarding the emergence percentage (E%), it was observed that treatments inoculated with AMFs were statistically different from control, with higher values, which were 70.48% for treatment with *G. margarita* + *G. rosea* and 73.15% for treatment with *R. clarus*, with no statistically significant difference between treatments, while the emergence percentage for control was only 51.11%.

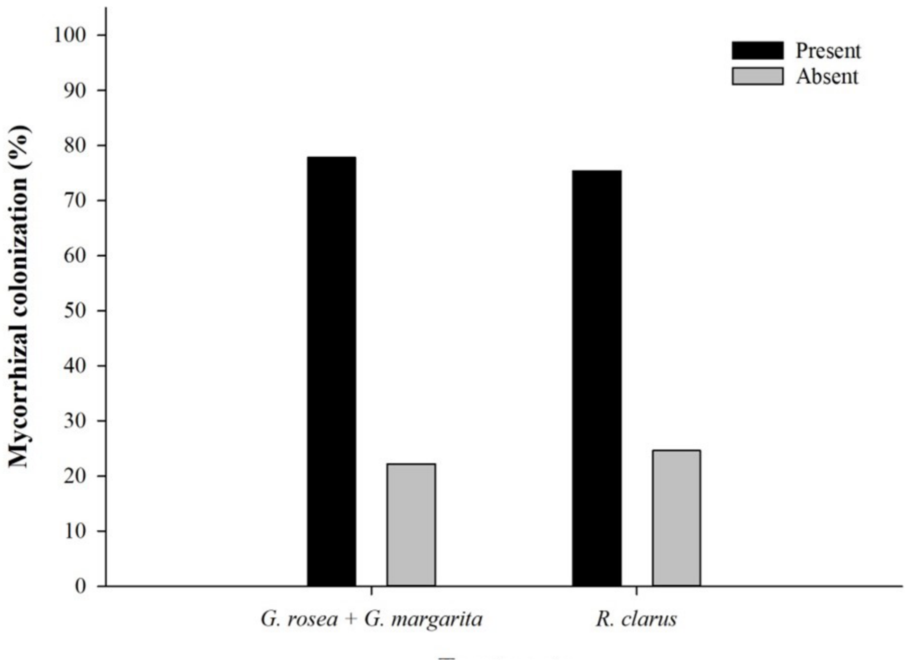

Figure 1: Percentage of papaya (*Carica papaya* L.) seedling roots colonized by mycorrhizal fungi. GR+GM: Inoculated with Arbuscular Mycorrhizal Fungi (AMF) *Gigaspora rosea* + *Gigaspora margarita*; RC: Inoculated with Arbuscular Mycorrhizal Fungi (AMF) *Rhizophagus clarus*.

Table 1: Emergence percentage (E%), Emergence Speed Index (ESI) and Mean Emergence Time (MET) of papaya seedlings inoculated with different arbuscular mycorrhizal fungi

TREATMENTS	E (%)	ESI	MET (days)
CONTROL	51.11 b*	0.81 c	19.32 a
GR+GM	70.48 a	1.33 b	16.39 b
RC	73.15 a	1.66 a	13.87 c
General average	65.68	1.30	16.36
CV (%)	37.90	41.87	11.80

* Means followed by different letters in the column differ statistically by the Tukey's test at 5%. GR+GM (*Gigaspora rosea* + *Gigaspora margarita*); RC (*Rhizophagus clarus*).

Paixão *et al.* (2020) worked with cattle manure and fertilizer in the emergence and initial development of papaya seedlings from the Formosa group and obtained emergence percentage of 77% for the control group, showing that, among other factors, the type of substrate used influences plant development (Melo *et al.*, 2015).

Regarding ESI, treatment with *Rhizophagus clarus* differed statistically from the other treatments, with value of 1.66; however, treatment with *Gigaspora rosea* + *Gigaspora margarita*, with value of 1.33, differed statistically and positively from control and negatively from *Rhizophagus clarus*, and control treatment differed statistically with the lowest emergence speed index of 0.81, evidencing the potential use of AMFs in the production of papaya seedlings.

The Emergence Speed Index of papaya is quite variable in literature. Melo *et al.* (2015), found ESI values of 1.21, which are closer to values found in the present study, which showed general average value of 1.3. In this case, lower ESI values are related to seed vigor, which is important to indicate that they have good germination capacity, shorter time from sowing to emergence, as well as satisfactory development, among other factors (Vale *et al.*, 2020).

Regarding the Mean Emergence Time (MET), it was observed that papaya seeds inoculated with AMF had MET significantly reduced in relation to control, but those inoculated with *Rhizophagus clarus* had the shortest mean

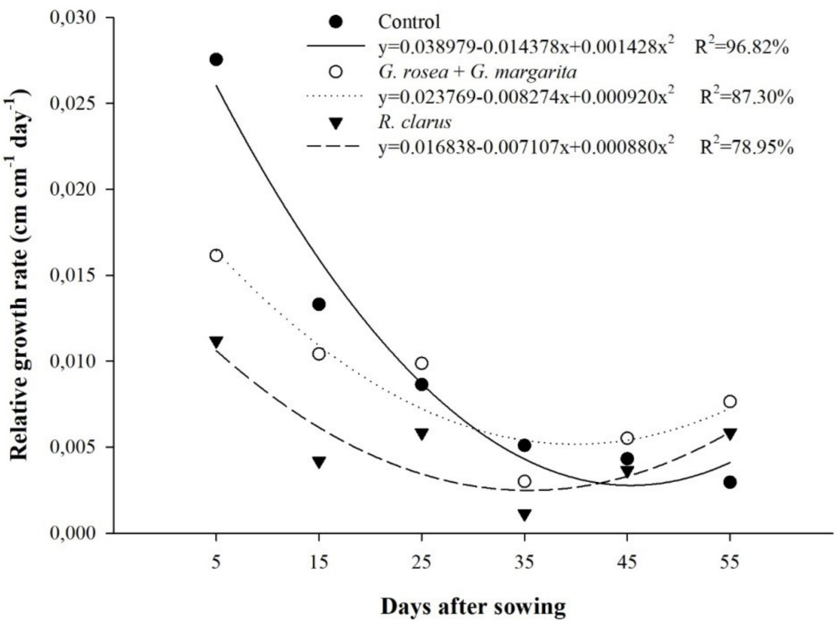

Figure 2: Relative growth rate of treatments Control, *Gigaspora rosea* + *Gigaspora margarita* and *Rhizophagus clarus*, of Tainung 1papaya plants during the seedling growth phase.

Table 2: Shoot length (SL), longest root length (RL), stem diameter (SD), fresh mass (FM) and dry mass (DM) of papaya seedlings inoculated with different arbuscular mycorrhizal fungi

TREATMENTS	SL (cm)	RL (cm)	SD (mm)	FM (g/plant)	DM (g/plant)
CONTROL	3.83 a*	13.87 a	1.39 a	3.64 ab	2.98 ab
GR+GM	3.68 a	13.92 a	1.45 a	3.77 a	3.16 a
RC	4.33 a	11.23 a	1.49 a	3.48 b	2.80 b
General average	3.95	13.01	1.44	3.63	2.98
CV (%)	19.28	44.69	12.39	6.60	11.90

*Means followed by different letters in the column differ statistically by the Tukey's test at 5%. GR+GM (*Gigaspora rosea* + *Gigaspora margarita*); RC (*Rhizophagus clarus*).

emergence time (MET), 13.87 days, significantly differing from the other treatments. Treatment with *Gigaspora rosea* + *Gigaspora margarita* obtained median result of 16.39 days, being statistically different from control (19.23 days).

Studies reveal several benefits in the use of AMF in the initial phase of the papaya crop, because in addition to influencing nutrition and growth, AMF reduces the time that seedlings remain in the nursery, reducing costs with inputs and labor, in addition to providing greater seedling vigor and survival after transplanting to the field, reducing additional costs with replanting (Begum *et al.*, 2019).

AMF also seem to play a significant role in increasing leaf chlorophyll content, photosynthesis rate, root, stem and leaf NPK content, increasing shoot biomass (Janeeshma & Puthur, 2020) and, consequently, increasing the growth of forest plant seedlings in the nursery compared to plants without mycorrhizal inoculation (Wang *et al.*, 2019).

The relative growth rate (RGR) is an indicator of the extent to which a species is using its photoassimilates for growth and is affected by environmental factors (Puglielli *et al.*, 2017). The rapid accumulation of material followed by smaller increment can be explained by the increase in intraspecific competition for the main environmental factors responsible for plant growth, such as light, nutrients and CO_2 diffusion.

Regarding the Relative Growth Rate (RGR) of papaya seedlings, it was observed that the behavior of RGR curves differs between treatments inoculated with mycorrhiza and control, as can be seen in Figure 2.

For seedling height at 60 DAS (Table 2), it could be verified that treatment inoculated with *R. clarus*, despite not being statistically different from the others, presented the highest means, with 4.33 cm, corroborating with Salles *et al.* (2019), that evaluating the production of papaya seedlings under different substrate compositions and different wavelengths within the nursery, observed, at 60 DAS, seedling height values between 3.0 and 4.0 cm at 66 DAS.

For variable root length (cm), no statistical difference between treatments was observed, obtaining experimental average of 13.01 cm. In this case, despite the lack of statistical difference, it was verified that treatment inoculated with *R. clarus* had the lowest average, 11.23 cm, contrary to previous parameters, but in accordance with literature. Silva *et al.* (2016), using different substrates, found average value of 10.5 cm, corroborating results found in the present work.

For variables fresh and dry mass, treatment with *Gigaspora rosea* + *Gigaspora margarita* showed higher values compared to *Rhizophagus clarus*, but when compared to control, values did not differ statistically in relation to fresh and dry matter in both treatments with mycorrhiza.

A balanced growth relationship between seedling height and diameter tends to promote plants with higher percentage shoot phytomass distribution, which makes seedlings more robust; however, Binotti *et al.* (2019), worked with levels of shading and plant growth regulator in the formation of *Schizolobium amazonicum* seedlings and concluded that compact plants have greater phytomass partition to roots, which may justify the results of inverse relationship between shoot development with the root system and total seedling mass found in the present study.

CONCLUSIONS

It was concluded that the inoculation of papaya seedlings with Rhizophagus clarus of AMF and Gigaspora rosea + Gigaspora margarita was effective, thus occurring the symbiotic process.

With inoculations with Rhizophagus clarus, and Gigaspora rosea + Gigaspora margarita, an increase in the emergence speed index (ESI) and a decrease in the mean time to emergence (MET) were observed in the seedlings after inoculation, in addition to an increase in the biometric characteristics of the seedlings, evidencing their beneficial use for a higher quality production of seedlings.

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