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STUDIES ON VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI OF PAPAYA IN BALLIA DISTRICT OF UTTAR PRADESH INDIA

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ABSTRACT

Papaya (*Carica papaya* L.) is one of the most cultivated fruits in Azamgarh (U.P.). Thus, increasing efforts to improve the crop efficiency have been carried out, being the study of quality seedling production of fundamental importance. Vesicular 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 the evaluate the interaction of papaya seeds treated with VAMF, in order to support management works on the corp. The experiment was installed in an agricultural greenhouse in a completely randomized design with three treatments: T0-control (no inoculuation); T1-inoculation of seeds with *Gigaspora rosea* + *Gigaspora margarita*; and T2-inoculation of seeds with *Rhizaophagus clars*, with 20 replicates, with each sowing cell being considered a replicate. Mycorrhizal colonization, seedling emergence and biometric indicates 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 of increase in biometric characteristics of seedling, evidencing its beneficial use for higher production.

KEYWORDS: *Carica papaya* L.; Vesicular Arbuscular Mycorrhizal Fungi (VAMF); Symbiosis; Seedling Quality; Sustainability

Vesicular Arbuscular Mycorrhizal Fungi (VAMF) penetrates the roots of plants to form a mutualistic symbiotic relationship. Mineral nutrients, mainly phosphorus, nitrogen and water are extracted from the soil via the extensive hyphal network and transferred to the plant. Organic carbon compounds are transferred to the VAMF in return. They are known to improve plant nutrient uptake, protect plants from pathogens and better against adverse environmental conditions, especially drought.

Vesicular Arbuscular Mycorrhizal Fungi can promote rapid increase in plant growth and contribute to better establishment of seedlings when transplanted to the field. In nursery; inoculation of these fungi can improve the plant growth, reducing the time for seedling production and protecting the plants against soil-borne pathogens.

Papaya (Carica papaya L.), belonging to the Caricaceae 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).

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

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 culture, 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 vesicular arbuscular mycorrhizal fungi (VAMF) can bring benefits to the production of quality papaya seedlings, since plants in symbiosis with VAMF undergo biochemical, physiological and molecular changes related to their defense system so that symbiosis is established (Garcia+Garrido and Ocampo, 2022), improving their tolerance to biotic and abiotic factors (Hu *et al.*, 2015; Hage-Ahmed *et al.*, 2019)

Thus inoculation with VAMF in the production phase of papaya seedling can become an alternative technology with potential for practical application. Therefore, the present study aimed to evaluate the effect

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of inoculation of different VAM fungi (VAMF) on papaya seeds, aiming at the formation of healthy seedlings in order to subsidize management and crop production studies.

MATERIALS AND METHODS

The arrearage wind speed in Ballia District U.P. (India) is 2.7 m/s with the maximum wind speed of around 10 m/s. The average ambient temperature remain 25.4^{0}C, Varies from 7.1^{0}C to 41.1^{0}C. The average relative humidity remain around 69.3%, varies from 16.1% to 100%. The station pressure varies from 100 hpa to 982 hpa, average around 1016 hpa. The average rainfall of district is 1031 cm. The selected coordinates i.e. lateral : 26°3'0" N, longitude : 83°13'0" E is found within the limit of Ballia District, in the state Uttar Pradesh, India.

For this experiment the treatments consisted of the species of Vesicular Arbuscular Mycorrhizal fungi (VAMF), previously identified as *Gigaspora rosea* + *Gigaspora margarita* and *Rhizophagus clarus* belonging to the collection from different areas of District Azamgarh (U.P.)

The experiment was conducted in a completely randomized design containing three treatments, namely: T0- no inoculation, with pots filled only with 500g soil commercial agricultural substrate not autoclaved; T1-incoluation with 20g the soil with *Gigaspora rosea* + *Gigaspora margarita* added to 480 g of autoclaved substrate; and T2-inoculation with 20g with soil with of *Rhizaophagus clarus* added to 480g of autoclaved substrate, with 20 replicates, with each pot considered an experimental unit. Inoculation was performed when the seeds were sown in the pots.

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 the length, in centimeters, every 10 days, using the formula used by Benincasa (2003)- RGR = (In R2–In R1)/ΔT where, In = 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 calipers and determined close to 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, weighting whole seedlings on scale with precious of 0.001g (1 mg), obtained 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°C, for 48 hours, until constant dry mass is obtained, measured on analytical scale with precision of 0.0001g, with results expressed in g seedling^{-1}.

In addition, in order to verify the efficiency of seed inoculation with VAMF the inoculated 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 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 table, 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 table.

RESULTS AND DISCUSSION

From results presented in 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, in generally, VAMF 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 infectively is not always correlated with the efficiency of symbiosis (Konvalinkova and 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 and Meek, 2020).

Regarding germination variable 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 VAMF were statistically different from control, with higher values, which 74.48% for treatment with *Gigaspora margarita* + *Gigaspora rosea* and 77.15% for treatment with *Rhizophagus clarus*, with no statistically significant difference between treatments, while the emergence percentage for control was only 54.11%.

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

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

Treatments	E(%)	ESI	MET (days)
Control (T0)	54.11 b*	0.88 c	20.32 a
Gigaspora rosea + *Gigaspora margarita* (T1)	74.48 a	1.36 b	18.39 b
Rhizophagus clarus (T2)	77.15 a	1.77 a	14.82 c
General average	65.68	1.30	16.36
CV (%)	37.90	41.87	11.80

*Mean followed by different letters in the column differs statistically by the Tukey's test at 5%. (*Gigaspora rosea* + *Gigaspora margarita*), (*Rhizophagus clarus*).

Regarding ESI, treatment with *Rhizophagus clarus* differed statistically from the other treatments, with value of 1.77; however, treatment with *Gigaspora rosea* + *Gigaspora margarita*, with value of 1.36, differed statistically and positively from control and negatively from *Rhizophagus clarus*, and control treatment differed statistically with the lowest emergency speed index of 0.88, evidencing the potential use of VAMF in the production of payaya seedlings.

The Emergency 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 value are related to seed vigor, which is important to indicate that they have good germination capacity, shorter time form sowing to emergence, as well as satisfactory development, among other factors (Vale *et al.,* 2020).

Regarding the Mean Emergency Time (MET), it was observed that papaya seeds inoculated with AMF had MET is significantly reduced in relation to control, but those inoculated with *Rhizophagus clarus* had the shortest Mean Emergency Time (MET), 14.82 days, significantly differing from the other treatments. Treatment with *Gigaspora rosea* + *Gigaspora margarita* obtained median result of 18.39 days, being statistically different from control 20.33 days.

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

VAMF also seems to play a significant role in increasing leaf chlorophyll content, photosynthesis rate, roots, 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 form growth and is affected by environment factors (Puglielli *et al.,* 2017). The rapid accumulation of material followed by smaller increment can be explained by the increase in instraspecific 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.

For seedling height at 60 DAS (Table 2), it could be verified that treatment inoculated with *Rhizophagus clarus*, despite not being statistically different from the others, presented the highest means, with 5.36 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 to 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 *Rhizophagus clarus* had the lowest average, 11.25 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.

Table 2: Shoot Length (SL) longest Root Length (RL), Stem Diameter (SD), Fresh Mass (FM) and Dry Mass (DM) of papaya seedling inoculated with different vesicular arbuscular mycorrhizal fungi

Treatments	SL (cm)	RL (cm)	SD (mm)	FM (g/plant)	DM (g/plant)
Control (T0)	3.83*	13.87a	1.39a	3.64ab	2.98ab
Gigaspora rosea + *Gigaspora margarita* (T1)	3.68a	13.92a	1.45a	3.77a	3.16a
Rhizophagus clarus (T2)	5.36a	11.25a	1.49a	3.48b	2.80b
General average	3.95	13.01	1.44	3.63	2.98
CV (%)	19.28	44.69	12.39	6.60	11.90

*Mean followed by different letters in the column differs statistically by the Tukey's test at 5%. *Gigaspora rosea* + *Gigaspora margarita*, *Rhizophagus clarus*).

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* seedling 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.

CONCLUSION

It was concluded that the inoculation of papaya seedling with *Rhizophagus clarus* of AMF and (VAMF) *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 Emergence Time (MET) were observed in the seedlings after inoculations, 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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