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Early growth of *Syzygium tripinnatum* (Blanco) Merr. seedlings inoculated with biofertilizers under nursery condition

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Abstract

The early growth of *Syzygium tripinnatum* (Blanco) Merr. seedlings applied with biofertilizer inoculants was evaluated under nursery condition at the Don Mariano Marcos Memorial State University, La Union, Philippines. This study generally aimed to recommend a biofertilizer inoculation protocol for *S. tripinnatum* seedlings for the production of its quality planting materials. Three treatments were used: T_0- Uninoculated (Control), T_1- Mycogroe, and T_2- Mykovam. Results revealed that the height, diameter and root, shoot, and seedling dry weights of the seedlings were significantly improved when applied with biofertilizer inoculants, particularly Mykovam biofertilizer. However, no significant differences were observed among all treatments for the number of leaves, sturdiness quotient, and root-shoot ratio. Based on the seedling quality index, a significant result was observed in favor of the inoculated seedlings. Regardless of treatments, the sturdiness quotient was relatively higher than the acceptable standard value; while lower values were obtained for all seedlings in terms of root-shoot ratio. Therefore, *S. tripinnatum* seedlings may be susceptible to wind, drought, and cold, and have low absorption and storage capacity of water which could produce worse response in the field. However, early growth of *S. tripinnatum* seedlings will likely improve when applied with biofertilizer inoculants, particularly Mykovam and undergone preconditioning activities like hardening before out planting.

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Introduction

The Philippines boasts of more than 300 species of edible fruits and nuts but only 20 species are cultivated commercially (Rodeo, 2016). The favorable climate and fertile soils of the country make it an ideal location for tropical fruit production. Promoting the local native fruits of the country that show huge market potentials may help increase income of the community especially farmers and their families who are into fruit production and processing.

Syzygium tripinnatum (Blanco) Merr. is one of the native fruit tree species in the Philippines, commonly known as *hagis,* under the family Myrtaceae and mostly found in the provinces of Sorsogon and Bicol. It has been listed by Coronel (2002) as one of the economically useful indigenous fruits of the Philippines. It is characterized as a medium-sized tree that grows up to 20 m high. It has thinner, chartaceous leaves, and longer peduncles compared to *Syzygium jambos* (Linnaeus) Alston var. jambos and has fruit that are red and ellipsoid when ripe (Jie and Craven, 2006). The present uses of *S. tripinnatum* include raw consumption of its ripe berry and being processed into wine (Coronel, 2002). Further, its ripe fruit has a juicy and sour taste that can also be processed into jam, jelly, or juice. Particularly, *S. tripinnatum* fruits that were processed into wine is one among the University's fruit-based wine products at the Don Mariano Marcos Memorial State University, Bacnotan, La Union.

In consideration, *S. tripinnatum* is a lesser-known native fruit tree species and often overlooked not because it is not as delicious and nutritious compared to other fruits, but due to its unfamiliarity. Very little literature about the silvics and silviculture of *S. tripinnatum* seedlings is available despite of various efforts of mainstreaming the conduct of studies for the different native tree species in the country. Coronel (2013) claimed that it is one of the fruit tree species that have not been studies before but is recognized as an underutilized fruit species with economic potential as new crops for processing. Thus, it is vital to have research and understanding about its growth performance to address enormous knowledge-gap on this particular species towards better production and utilization. Additionally, production of forest seedlings is one of the most important stages in the establishment of forest stands as it strongly affects forest yield. A successful, high yielding stand is closely dependent on the quality of planted seedlings, which should be capable of resisting adverse field conditions and grow into trees that are economically desirable (Binotto *et al.,* 2010).

Meanwhile, biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that aid crop plant nutrient uptake through interactions in the rhizosphere when applied through seed or soil. They speed up certain microbial processes in the soil, increasing the availability of nutrients in a form that plants can easily absorb. Increasing the population of beneficial microorganisms in the rhizosphere by biofertilizers is a strategy to ensure sustainability in soil fertility. The study used two kinds of biofertilizers, namely Mycogroe and Mykovam that are eco-friendly, productive, efficient, and cheaper to marginal and small farmers over chemical fertilizers.

As discussed by Brown *et al.* (2016), Mykovam is a soil based biofertilizer containing spores, infected roots propagules and vesicular arbuscular mycorrhizal (VAM) fungi that increases the effectiveness of nutrient absorption. A positive side effect of Mykovam inoculation is the increased tolerance to pathogens, environmental stresses such as water deficit and heavy metal exposure. Application of the product has the potential to replace 60-85% of the commercial fertilizers used in cultivation. While Mycogroe is a biofertilizer comprised of spores from ectomycorrhizal fungi collected during rainy season under pine and eucalyptus plantations. The product is able to reduce application of chemical fertilizer by 60-85%. It facilitates better water and nutrient uptake of plants and prevention of infection from plant pathogens and as well as increased plant tolerance against drought and heavy metals.

This study aimed to determine the response of *S. tripinnatum* seedlings to Mycogroe and Mykovam under nursery condition in terms of height and diameter growth, root, shoot and seedling dry weight, root-shoot ratio, production of leaves, sturdiness quotient, and seedling quality index. Generally, the results of the study may serve as a guide to the production of quality *S. tripinnatum* planting materials using effective biofertilizer inoculants. Furthermore, this study may contribute to the promotion of *S. tripinnatum* and its fruit-processed products such as hagis wine, especially in the Province of La Union as a potential source of income for the community.

Materials and methods

Research Design

The study was laid out following the Completely Randomized Design (CRD) with three treatments replicated three times. The treatments used were the T_0- Uninoculated (Control), T_1- Mycogroe, and T_2- Mykovam. 24 seven-month-old *S. tripinnatum* seedlings were utilized per plot making a total of 216 seedlings for the whole study occupying 40m^2.

Sample Preparation

S. tripinnatum seeds were collected at the Agroforestry Nursery of DMMMSU – College of Agroforestry and Forestry. Mycogroe and Mykovam were purchased at the National Institute of Applied Microbiology and Biotechnology (BIOTECH) at the University of the Philippines Los Baños, College, Laguna. *S. tripinnatum* seeds were sown in seed beds and were transplanted into 5”x7” polyethylene plastic bags one month after germination.

The soil medium used was composed of sterilized garden soil and carbonized rice hull with the ratio of 2:1. Moreover, the application of biofertilizer inoculants was done based on the recommended rate of one capsule per seedling for Mycogroe and 2.5 grams per seedling for Mykovam at about four centimeters deep and two centimeters away from the base of the seedlings. Watering was done every other day (every 6-7 A.M.), while weeding was done as needed.

Data Gathered

Diameter (mm)

The initial diameter, final diameter, and diameter increment were obtained by measuring the seedlings at their root collar using a vernier caliper.

Height (cm)

The initial height, final height, and height increment were obtained by measuring the seedlings from the root collar up to the tip of the apical bud using a meter stick.

Number of leaves

Initial number of leaves, additional number of leaves and final number of leaves were gathered by counting the number of leaves of the seedlings just after the application of biofertilizers, after one and two months from inoculation at the termination of the study, respectively.

Root, shoot, and seedling dry weight (g)

Dry weights were determined by getting five sample plants from each plot through destructive sampling. Samples were washed in running water to remove attached soil particles in the roots. The roots and shoots were separated by cutting at the root collar. Seedling height and root collar diameter of the samples were again obtained. Roots and shoots were then separately wrapped with paper for oven drying for 72 hours at 105°C ± 5. After which, samples were placed in a desiccator for two hours to let the weights and temperature of the samples settle and cooled, respectively. After which, the roots and shoots were weighed separately and recorded. Seedling dry weight was obtained by adding the dry weight of the root and shoot of each sample.

Root-shoot ratio

This was determined by dividing the oven dry weight of roots by the oven dry weight of shoots of the seedlings (Takoutsing *et al.,* 2013).

Sturdiness Quotient

This was determined by dividing the height (cm) of the seedlings with the diameter (mm) taken at root collar level (Takoutsing *et al.,* 2013).

Seedling Quality Index

This was determined following the Dickson's Quality Index (DQI) developed by Dickson *et al.* (1960) which was calculated as:

$$\text{Quality index} = \frac{\text{Seedling dry wieght (g)}}{\frac{\text{Height (cm)}}{\text{Root collar diameter (mm)}} + \frac{\text{Shoot dry wieght (g)}}{\text{Root dry weight (g)}}}$$

Data Analysis

Analysis of variance (ANOVA) in Completely Randomized Design (CRD) was used to compare the differences of all the data gathered. Also, Tukey's Honestly Significant Difference test was used to determine the significant differences between and among treatment means/percentages. All statistical analyses were generated using R-Studio v. 2022 (R Core Team, 2022).

Results and discussion

Morphological Characteristics

Diameter (mm)

The influence of mycorrhizal inoculants on the diameter of *S. tripinnatum* seedlings revealed that only during the first month after inoculation showed significant result, displaying a more favorable effect of the two mycorrhizal inoculants compared to the uninoculated (control) ones, as presented in Table 1.

Table 1. Effects of mycorrhizal inoculation treatment on diameter growth of *S. tripinnatum* seedlings under nursery condition.

Treatments	Diameter (mm)		
	1-mo AI*	2-mo AI⁺	3-mo AI⁺
Uninoculated	4.93b	5.75a	6.40a
Mycogroe	5.28a	6.08a	6.81a
Mykovam	5.38a	6.15a	6.91a

AI- After inoculation; * - significant at $p < 0.05$; $^{+}$ - not significant; treatment means in each column with the same letters are not significantly different from each other using Tukey's test at $p < 0.05$.

Specifically, Mykovam and Mycogroe with a diameter of 5.38mm and 5.28mm, respectively, were found comparable with each other, while the uninoculated (control) seedlings obtained significantly lower diameter of 4.93mm. Moreover, seedlings inoculated with Mykovam obtained the largest diameters during first, second and third month after inoculation with 5.38, 6.15, and 6.91 (mm), respectively. While the smallest diameter was obtained by the uninoculated seedlings with 4.93, 5.75, and 6.40 (mm) during first, second and third month after inoculation, respectively.

Height (cm)

The monthly height growth of *S. tripinnatum* seedlings was found to be significantly affected with mycorrhizal inoculants under nursery condition (Table 2). Statistical analysis revealed that seedlings applied with Mykovam attained the tallest height during the whole duration of the study and was found to be significantly greater than the uninoculated seedlings. Specifically, a height of 42.33cm was attained by the seedlings inoculated with Mykovam, while 40.78 and 37.26 (cm) were attained by the Mycogroe-inoculated and uninoculated seedlings during first month after inoculation, respectively.

Moreover, a height of 44.31cm was attained by the seedlings inoculated with Mykovam during second month after inoculation, while 42.60 and 39.71 (cm) were recorded from the Mycogroe-inoculated and uninoculated seedlings, respectively. On the third month after inoculation, seedlings inoculated with Mykovam attained a height of 47.35cm, while the Mycogroe-inoculated and uninoculated seedlings attained 44.81 and 42.38 (cm), respectively. The effects of both mycorrhizal inoculants were found comparable with each other, however, no significant difference was observed between the Mycogroe-inoculated seedlings and uninoculated seedlings during the second and third month after inoculation.

Table 2. Effects of mycorrhizal inoculation treatment on height growth of *S. tripinnatum* seedlings under nursery condition.

Treatments	Height (cm)		
	1-mo AI*	2-mo AI*	3-mo AI*
Uninoculated	37.26b	39.71b	42.38b
Mycogroe	40.78a	42.60ab	44.81ab
Mykovam	42.33a	44.31a	47.35a

AI - After inoculation; * - significant at $p < 0.05$; treatment means in each column with the same letters are not significantly different from each other using Tukey's test at $p < 0.05$.

The data revealed that Mykovam biofertilizer showed better results than Mycogroe biofertilizer. The favorable effect of Mykovam biofertilizer was also recorded in the previous studies on *J. curcas* (Aggangan *et al.,* 2013a; 2019a), *T. cacao* (Aggangan *et al.,* 2019b), *S. cuminii* (Surakshitha and Kumar, 2015), and *V. parviflora* (Castañeto, 2001). Specifically, the previous studies observed that there was a higher increase in stem diameter and height on the four various plant species. Surakshitha and Kumar (2015) stated that application of arbuscular mycorrhiza like those present in Mykovam helps in nutrient and water uptake, and maintenance of good physical and chemical properties of the soil. They believed that this might be the reason for the increase in height and diameter of *S. cuminii* seedlings. Likewise, Aggangan *et al.* (2013b) specified that Mykovam biofertilizer is very effective in increasing yield and survival of agricultural crops, forest species, horticultural plants, forage crops and fruit crops.

Number of Leaves

The influence of mycorrhizal inoculants on the number of leaves of *S. tripinnatum* seedlings was observed to be comparable among each other, as shown in Table 3. Nevertheless, the greatest number of leaves was obtained by seedlings applied with Mykovam that attained an average number of 46.22, 45.19, and 58.60 during first, second and third month after inoculation, respectively. On the other hand, the uninoculated seedlings attained the lowest number of leaves for the whole duration of the study with an average number of 41.78, 43.03, and 53.39 during first, second and third month after inoculation, respectively.

Table 3. Effects of mycorrhizal inoculation treatment on the number of leaves of *S. tripinnatum* seedlings under nursery condition.

Treatments	Number of Leaves		
	1-mo AI$^+$	2-mo AI$^+$	3-mo AI$^+$
Uninoculated	41.78a	43.03a	53.39a
Mycogroe	44.13a	44.99a	57.91a
Mykovam	46.22a	45.19a	58.60a

AI- After inoculation; $^+$ - not significant; treatment means in each column with the same letters are not significantly different from each other using Tukey's test at $p < 0.05$.

Despite Mykovam showing no significant difference among other treatments, its favorable performance than Mycogroe and uninoculated was relatively evident. The result of the study corroborates with the findings of Surakshitha and Kumar (2015) on their study on *S. cuminii* that revealed greater number of leaves in seedlings applied with Mykovam. Furthermore, Mykovam biofertilizer also exemplified more favorable performance in Banana var. lakatan, compared to other biofertilizers as reported by Aggangan *et al.* (2013b) and Elleva *et al.* (2018). They observed greater leaf area, number of leaves and overall plant growth for Banana var. lakatan applied with Mykovam biofertilizer. According to Aggangan *et al.* (2013a), the better growth of Mycorrhizal seedlings than uninoculated seedlings in terms of height, diameter, and number of leaves may be attributed to the extraradical hyphae produced by mycorrhizal fungi ramifying beyond the root hair zone. These extraradical hyphae are effective in increasing the root absorptive area of plants. The diameter of mycorrhizal mycelia is smaller, thus the transport of nutrients from the soil to the plant is faster through these structures than nutrient transport via root hairs. Thus, it is possible that inoculated seedlings were better supplied with nutrients taken up by the extraradical hyphae, resulting in improved growth.

Root, shoot, and seedling dry weight (g)

The root, shoot, and seedling dry weights of *S. tripinnatum* seedlings were found significantly affected with mycorrhizal inoculants during three months after inoculation under nursery condition, as shown in Fig. 1. The result showed that the mycorrhizal seedlings comparatively manifested heavier root, shoot, and seedling dry weights than the uninoculated ones. Particularly, the seedlings inoculated with Mycogroe obtained the highest root dry weight with 3.09g, while seedlings inoculated with Mykovam showed the heaviest shoot and seedling dry weights having 7.75g and 10.82g, respectively. On the other hand, the uninoculated seedlings were found comparable with the Mycogroe-inoculated seedlings in terms of the shoot and seedling dry weights.

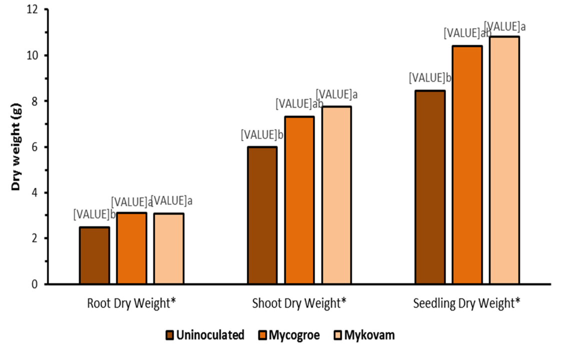

Fig. 1. Effects of mycorrhizal inoculation treatment on the root dry weight shoot dry weight, and seedling dry weight of *S. tripinnatum* seedlings under nursery condition. *- significant at $p < 0.05$. Treatment means in each bar with the same letters are not significantly different from each other using Tukey's HSD test at $p < 0.05$.

The inoculation of Mykovam being the most favorable in promoting the shoot and seedling dry weights of *S. tripinnatum* conforms with the findings of Gamage *et al.* (2004) wherein *S. firmnum* having only moderate arbuscular mycorrhizal colonization but recorded a high total dry mass. Also, it was further supported with the reports of Aggangan *et al.* (2019b) on *T. cacao* mycorrhizal seedlings that obtained heavier total dry weight than the non-mycorrhizal seedlings. Likewise, *V. parviflora* seedlings (Castañeto, 2001) and cultured Banana var. lakatan (Aggangan *et al.*, 2013b) applied with Mykovam biofertilizer showed higher shoot biomass and total plant biomass compared to the uninoculated seedlings.

This indicates that the application of Mykovam biofertilizer will likely increase the dry weight of *S. tripinnatum* seedlings. In relation to root dry weight, seedlings applied with Mycogroe biofertilizer were relatively higher with other treatments, although no significant difference was observed to the other biofertilizer treatment used. The result of this study corroborates with the findings of Moon and Aggangan (2018) on the response of *E. pellita* as applied with different mycorrhizal inoculants which recorded 85% increase in root dry weight for seedlings applied with Mycogroe compared to only 15% increase recorded for seedlings applied with Mykovam.

Root-shoot ratio

The effect of mycorrhizal inoculation treatment on the root-shoot ratio of S. tripinnatum seedlings under nursery condition was shown in Fig. 2. The result showed that in all treatments, the root-shoot ratios were relatively lower than the standard value. The highest root-shoot ratio was obtained by the seedlings inoculated with Mycogroe with 0.43, followed by uninoculated seedlings (0.42) and Mykovam-inoculated seedlings (0.40). However, no significant difference was observed among treatments. According to Jaenicke (1999), a root-shoot ratio between one and two is favorable and considered as optimal value. The root-shoot ratio is an important measure for seedling survival as it measures the balance between the transpiration area (shoot) and the water absorbing area (root) of the seedlings (Haase, 2008). A high root-shoot ratio indicates high absorption and storage capacity of water, which is of advantage especially in the condition of limited moisture in the soil (Jaenicke, 1999; Takoutsing *et al.*, 2013). As a result, *S. tripinnatum* seedlings might have low seedling survival potential especially to areas with limited water supply, in consideration with root-shoot ratio as a tool in the assessment of seedling quality. However, Lin *et al.* (2018) reiterated that younger plants generally have smaller ratios compared to older plants.

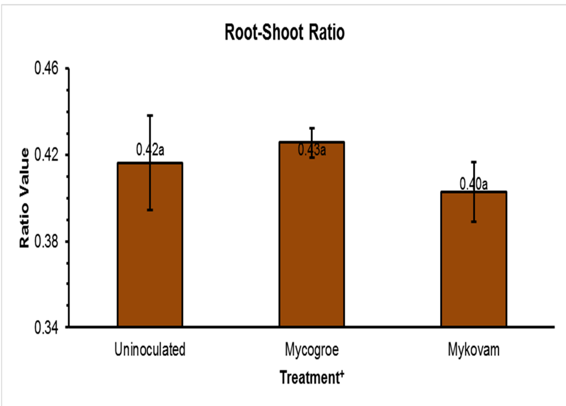

Fig. 2. Effects of mycorrhizal inoculation treatment on the root-shoot ratio of *S. tripinnatum* seedlings under nursery condition. + - not significant. Treatment means in each bar with the same letters are not significantly different from each other using Tukey's HSD test at $p < 0.05$.

Sturdiness Quotient

The most favorable sturdiness quotient was attained by the uninoculated seedlings for the whole duration of the study, although the recorded values showed no significant difference observed among treatments as shown in Fig. 3. Specifically, the uninoculated seedlings attained a value of 7.6 on the first month, 6.9 on the second month, and 6.6 on the third month after inoculation. While the highest value was attained by seedlings applied with Mykovam, attaining an average values of 7.9, 7.2, and 6.9 during the first, second, and third month after inoculation, respectively.

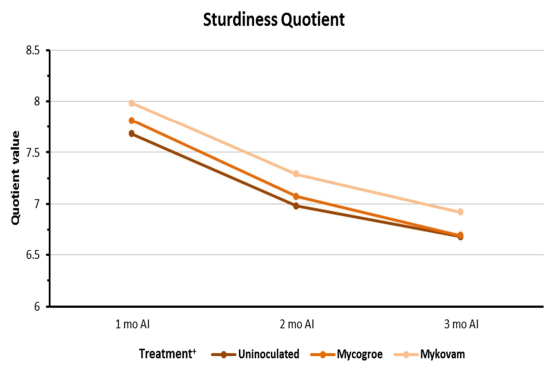

Fig. 3. Effects of mycorrhizal inoculation treatment on the sturdiness quotient of *S. tripinnatum* seedlings under nursery condition. + - not significant.

Regardless of treatments, the result of sturdiness quotient was relatively higher than the acceptable standard value that is less than six (Jaenicke, 1999). According to Jaenicke (1999) and supported by Luis *et al.* (2004), a sturdiness quotient higher than six indicates a very slender seedling which is susceptible to wind, drought, and cold.

Plants with high sturdiness quotient could produce worse response in the field. Therefore, a lower chance of survival can be expected for *S. tripinnatum* seedlings, especially when planted in windy and dry sites. It can also be observed in this study that the seedlings will tend to decrease their sturdiness quotient as they get mature which may indicate an improving sturdiness condition. In consideration, it is suggested to outplant seedlings with better sturdiness quotient to ensure lower rate of mortality in the field.

Seedling Quality Index

The seedling quality index values obtained from *S. tripinnatum* seedlings during three months after mycorrhizal inoculation under nursery condition was shown in Fig. 4. The results of the study revealed a significantly higher seedling quality index in seedlings inoculated with biofertilizers compared to the uninoculated ones. According to Binotto *et al.* (2010), seedling quality index was considered as a promising integrated measure of morphological traits which incorporates three parameters such as seedling dry mass, sturdiness quotient, and root-shoot ratio. It is also a useful indicator for seedling quality, seedling selection (Lin *et al.,* 2018), as well as plantation performance (Bayala *et al.,* 2009). Additionally, Lin *et al.* (2018) stated that a higher seedling quality index indicates a more desirable phenotype and better seedling vigor, or robustness associated with a balanced distribution of seedling biomass. Based on the result of this study, *S. tripinnatum* seedlings applied with Mykovam biofertilizer obtained the highest seedling quality index value among other treatments, showing significant difference from the uninoculated seedlings.

Fig. 4. Seedling quality index values obtained from *S. tripinnatum* seedlings, three months after mycorrhizal inoculation, under nursery condition. * - significant at $p < 0.05$. Treatment means in each bar with the same letters are not significantly different from each other using Tukey's test at $p < 0.05$.

Conclusion

The early growth of *Syzygium tripinnatum* (Blanco) Merr. Seedlings applied with biofertilizer inoculants under nursery condition was evaluated in this study.

The study found that the two biofertilizers (Mycogroe and Mykovam) applied have positive effects to the early growth of *S. tripinnatum* seedlings, especially the Mykovam biofertilizer, as they generally promoted better growth performances than the uninoculated. On the other hand, the result also revealed that *S. tripinnatum* seedlings were observed to be less sturdy making them more vulnerable when planted in areas that are frequently exposed to strong winds and with limited water supply. Based on the findings of the study, it is relatively relevant to consider the conduct of preconditioning activities like hardening before outplanting in order to increase the survival rate of *S. tripinnatum* in the field as well as the application of biofertilizers like Mykovam during the seedling stage of *S. tripinnatum* to promote early growth performance under nursery condition.

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