

INFLUENCED OF BOTTOM HEAT TREATMENT AND TYPES OF CUTTING ON THE REGENERATION OF EUGENIA (*Eugenia myrtifolia* L.) STEM CUTTINGS

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Abstract

Eugenia (*Eugenia myrtifolia* L.) is one of the most demanded ornamental plant in landscaping industry but the availability of planting material of this plant is limited due to its difficulty in propagating by stem cutting. Thus, this study was conducted to: (1) determine the influence of bottom heat on the regeneration of *Eugenia* stem cuttings; (2) determine the influence of type of cutting on the regeneration of *Eugenia* stem cuttings; and (3) evaluate the interaction effect of the type of cutting and bottom heat application on the regeneration of *Eugenia* stem cuttings. This was conducted at NwSSU of San Jorge Campus, San Jorge, Samar. The experiment was laid-out in 2 x 3 factorial in split-plot arranged in RCBD with three replications. The rooting medium temperature manipulation (B1- with bottom heat and B2- without bottom heat) was assigned in the Main-plot while type of cuttings (T1-with no axillary shoot, T2- with single axillary shoot, and T3- with two axillary shoot) in the sub-plot. Regardless of the type of cuttings, bottom heat application did not significantly influenced all cutting regeneration parameters evaluated but significantly affected some horticultural characteristics of the successfully regenerated cutting 30 days after potting. Application of bottom heat significantly promoted shoot production both in terms of number of shoot and size of leaves of successfully regenerated cuttings. The type of cutting significantly affected the percent leaf retention and rooting. Cuttings without axillary shoot retained more leaves and gave higher rooting percentage than cuttings with 2 axillary shoots. Successfully regenerated cuttings with 1 and 2 axillary shoots retained had bigger leaves 30 days after potting than cuttings without axillary shoot. There was no significant interaction effect of rooting medium temperature manipulation and type of cuttings on the regeneration of *Eugenia* stem cuttings and the performance of regenerated cuttings after potting

Key words: *Eugenia myrtifolia*, stem cuttings, bottom heat, type of cuttings, regeneration

INTRODUCTION

Eugenia (*Eugenia myrtifolia* L.) is among the foliage plants popularly used as houseplants and landscaping due to its dense and attractive foliage. It is commonly propagated by vegetative means because seed production is irregular (Toussaint et al., 1991). Commonly, it is propagated by stem cuttings, however at present propagators seldom achieve high percentage rooting because it is one of those considered difficult to root species. Rooting of *Eugenia* stem cuttings is not easy as these are considered dormant which need long time to root compared with other species (McMullen, 2011).

There are several factors that determine success of plant propagation by cuttings including, plant factors (species/varieties, type of cuttings, physiological maturity of the plant and cuttings), environmental factors (rooting medium temperature, medium moisture content, relative humidity and light), and treatment of cuttings (root inducer application, cutting storage etc.) (Hartman et al., 1990). Among the above factors, it was believed that rooting medium temperature and type of cuttings are among those affecting regeneration of *Eugenia* cuttings. For example, Lebrun (1998) revealed that *Eugenia* cuttings rooted in non-heated substrate (average of 16 °C) failed to root while those in heated substrate (average of 22±0.5 °C) gave 75% rooting on the best substrate. In contrast, Chong (2003) expressed that high bottom heat temperature at 21±2 °C caused more death of evergreen cuttings due to rotting than that of low bottom heat temperature of 12±2 °C. Mitchell (2012) pointed out that bottom heat application increases the root weight of the *Pinus patula* and *Pinus ellioti* x *Pinus caribaea* stem cuttings even in the warmest months with an optimum temperature of the rooting media ranging between 25 °C and 28 °C.

Regarding the type of cuttings, retention of more leaves/branches in cuttings have promoted rooting of *Cotinus coggygria* cv. Royal Purple (Cameron et al., 2005), Lavender (*L. dentate*) (de Bona & Biasi, 2010), Hibiscus (Ryan, 1958), *Terminalia spinosa* (Newton et al., 1992) and bitter melon (Malik et al., 2012). In contrast, in avocado cuttings, higher leaf retention gave negative effects in rooting due to the presence of high Mn (activator of auxin oxidase) that lowers the accumulation of auxin at cutting base and accelerates leaf shedding (Reuveni & Raviv, 1980;

Samish, 1957). Likewise, Hunter et al. (1997) pointed out that higher leaf retention has negative effects on the rooting of cuttings as the shoot apical meristem triggers metabolic process that favors shoot development rather than the root initiation process. Furthermore, Hartmann (2011) revealed that leaf retention had no effects on the rooting performance of cuttings of many crops species.

Due to limited available information regarding the rooting of Eugenia, stem cuttings the study was conducted to generate information that could be used in designing cutting propagation protocol for Eugenia, hence this was conducted to: determine the influence of bottom heat on the regeneration of Eugenia stem cuttings; determine the influence of type of cutting on the regeneration of Eugenia stem cuttings; and evaluate the interaction effect of the type of cutting and bottom heat application on the regeneration Eugenia stem cuttings.

MATERIALS AND METHODS

A. Treatments and experimental design

The experiment was laid out following a 2 x 3 factorial split-plot arranged in RCBD with three replications and having twenty (20) sample cuttings per treatment per replication. The following treatments were evaluated:

Main-plot (Rotting medium Temperature Manipulation)

B₁- without bottom heat applied

B₂- with bottom heat applied

Sub-plot (Type of Cuttings)

T₁ . no axillary shoot

T₂ . with single axillary shoot retained

T₃ . with 2 axillary shoot retained

B. Construction of Propagation Chamber and Rooting Medium

Propagation box 1.5 m wide and 3 m long and 1 m high was constructed using lumber and bamboo frame and transparent UV treated polyethylene plastic as cover. For the rooting box, thermal roof insulator was laid at the bottom of the box to contain the rooting medium and to serve as insulator (Figs. 1a and 1b). Mixture of alluvial soil and finely chopped coconut husk was used as rooting medium. This was prepared by thoroughly mixing finely chopped coconut husk and alluvial soil at 1:2 (v/v) ratio. Before this was use to fill the rooting box, this was sterilized by heating over fire for 4 hours. The rooting box was half-filled with the sterilized rooting medium or to a medium thickness of about 10 cm.



Figure 1. The propagation chamber (a- frame, b- with polyethylene cover

C. Installation of Soil Heater and Beds Disinfection

The soil heater (Jumps Start, 6m) was installed following the manufacturers recommended procedures. The soil heater cable was arranged at the bottom of the rooting box with no two sections closer than 16 to 20 cm to avoid having cold spots in the medium (Fig. 2a). After the soil heating cable was laid out, the rooting box was disinfected by spraying zonrox solution (Fig. 2b). The box was then uniformly covered with about 10 cm thick rooting medium (Fig. 2c).



Figure 2. Soil heater installation in the propagation bed (a- lay-outing of cable; b- disinfecting rooting bed by chlorine solution spray c- rooting with heater cable at the bottom uniformly covered with rooting medium)

D. Preparation of Mother Plant

The sample stem cuttings used in the experiment were taken from healthy and pest free *Eugenia* mother plants planted inside the NwSSU of San Jorge Campus.

E. Preparation of Stem Cuttings

Collection of *Eugenia* cuttings was done early in the morning to ensure that the cuttings have high moisture content. Healthy stems were selected and were carefully cut using a sharp pruning shear. The harvested cuttings were immediately placed inside transparent polyethylene bag, sprinkled with water and then, the mouth of the bag was tightly closed. These were immediately brought to the propagation area. Approximately 10 to 15 cm long cuttings were prepared following the types indicated in the treatments (Fig. 3) as follows: T1- cutting without axillary shoot, T2- cutting with 1 axillary shoot retained and T3- with 2 axillary shoots retained. The 2 basal pair of leaves were removed to facilitate staking. These were bundled and were labelled according to the assigned treatments.

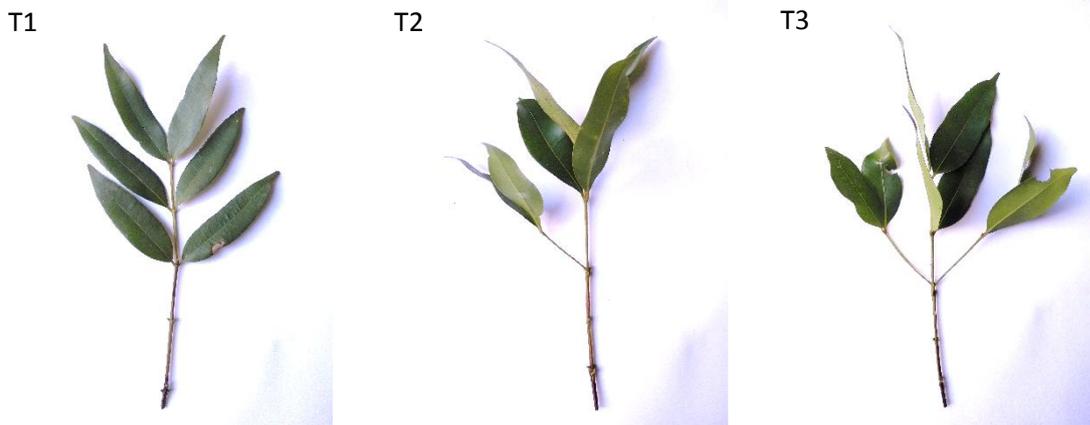


Figure 3. Type of *Eugenia* stem cuttings used in the experiment. (T1-cutting without axillary shoot retained; T2- cutting with 1 axillary shoot retained; T3- cutting with 2 axillary shoot retained)

F. Treatment of Cuttings with Naphthalene Acetic Acid

The basal ends of cuttings used in the experiment were uniformly dipped in pure Hormex solution with 2, 400 ppm NAA for 15 minutes.

G. Staking of Cutting

The rooting medium was moistened first with clean tap water before inserting the individual cuttings. These were vertically inserted by burying the first 2 basal nodes into the medium followed by slight pressing of the medium around the base to have good stem and medium contact. These were sprayed with fungicide solution (Dithane M-45) at 1tbsp/gallon of water right after staking and at biweekly interval thereafter.

H. Care and Maintenance

Regular RH checking was done to ensure uniform level of humidity (>85%) throughout the entire propagation period. To maintain the desired RH, fine water spray was applied using knapsack sprayer with the nozzle adjusted to the finest level. Moreover, to prevent temperature build up inside the chamber the propagation chamber was placed under an overhead shade made of coconut leaves to reduce the light intensity to 50%. Watering of cuttings was done at 5 days interval by applying fine water spray to the rooting medium using knapsack sprayer. Bi-weekly spraying of Benlate (Benomyl) at 3 tbs/16 L of water was done to minimize disease infection. Furthermore, removal of dead cutting was regularly done throughout the rooting period. After 85 days from staking, the live cuttings were hardened by opening the propagation chambers for 4 hours during the morning for the first 2 days and 4 hours in the afternoon for the succeeding 3 days.

I. Planting of Successfully regenerated Cuttings

Thirty successfully rooted cuttings (ten per replication) were planted in plastic bags (size containing a medium composed 3:1 v/v garden soil and carbonized rice hull) These were placed under partial shade during the first 15 days and then were exposed to full sunlight thereafter until 30 days. Watering was done regularly to keep medium moist.

J. Data gathered

The following data were gathered:

1. Environment inside the propagation chamber- the rooting medium temperature, air temperature and RH were monitored using hygrometer (OEM) at 6 AM, 2 PM and 6 pm. Rooting medium temperature was monitored daily for 10 days while air temperature and RH inside the chamber were monitored at 6 am, 2 pm and 6 pm daily for 90 days.
2. Cutting Regeneration Parameters- the following parameters were gathered 90 days after staking
 - a. Percent leaf retention- the initial and final (90 days after staking) number of leaves of each cutting were recorded and was expressed in percentage.
 - b. Percent rooting - the cuttings that successfully produced roots were counted and the data was expressed as percentage of the number of the total number of samples.
 - c. Number of primary roots/cutting – the primary roots produced by the cutting were counted.
 - d. Length of 2 longest primary roots- was measured using a ruler.
 - e. Diameter of adventitious roots- the diameter at the broadest portion of the sample roots used in d-parameter was measured using a vernier caliper.
 - f. Fresh and oven dried weight of roots- the fresh root weights of 5 sample cuttings were detached from the cuttings, washed with water, air dried for 30 minutes and then were weighed using platform balance. The samples were then oven dried at 70 ° C for 3 days, allowed to cool off for 1 hour and then were weighed using platform balance.
 - g. Root sugar and starch contents- sample roots used in dry weight determination were ground using Willey mill and then were submitted to the Central Analytical Services Laboratory of the Philippine Root Crops Research and Training, Visayas State University for sugar and starch analyses.
3. Horticultural characteristics of successfully regenerated cuttings 30 days after planting. The following parameters were gathered to evaluate the performance of the regenerated cuttings after planting.
 - a. Length of newly developed shoots- the length of all newly developed shoots were measured from the point of attachment to the tip by using ruler.
 - b. Number of newly formed leaves- all new formed leaves per cutting were counted.
 - c. Number of axillary shoots –all newly developed axillary shoots produced by each cutting were counted
 - d. Length and width of leaves – the length of 5 sample leaves from the newly developed leaves were measured from the base to the tip of the lamina. The width was taken at the broadest part of the leaf lamina.

K. Data analysis

Data analysis was done using the Statistical Tool for Agricultural Research (STAR), Plant Breeding Genetics and Biotechnology Biometrics and Breeding Informatics, version 2.0.1 (2014). Treatment means were compared using Least Significance Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSIONS

Environmental Conditions Inside the Propagation Chamber

The daily temperature of rooting medium with and without bottom heat monitored at 6:00 AM, 2:00 PM and 6:00 PM during the 10 –day propagation period is shown in Figures 4a, 4b and 4c. The temperature of the rooting medium applied with bottom heat was about 1.65 °C higher than that of the rooting medium without bottom heat treatment and about 2.03 °C higher than the air temperature. The average daily temperature of rooting medium with bottom heat, without bottom heat and air temperature throughout the 10-day propagation period were 27.6, 25.5 and 25.9 °C, respectively. The rooting medium temperature in both treated and non-treated rooting medium were within temperature considered ideal for warm climate species cuttings. Dykeman (1976) and Kester (1970) claimed that the optimum rooting medium temperature for propagation of warm-climate species is between 25 °C to 32 °C. Furthermore, McGroaty (2015) pointed out that the soil temperature should be higher by at least 2 °C as compared to the air temperature to promote adventitious root initiation.

Likewise, the average relative humidity inside the propagation chamber at 6 am was 98.7%, 93.58 at 2 pm and 98% at 6 pm. The average daily RH during the 12- week propagation period was 96.78%. A humid condition (>85% RH) that is conducive to rooting (Hartmann et al., 1997) was maintained inside the chamber throughout the 90-day propagation period (Fig. 5).

Soil and Air temperature inside the chamber

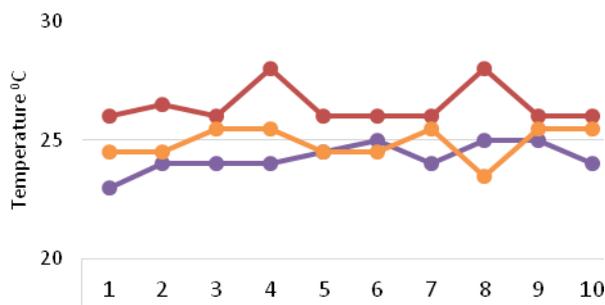


Figure 4a. Tenth day soil and air temperature

recorded at 6:00 AM

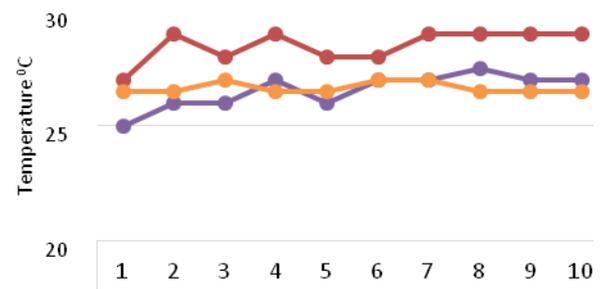


Figure 4b. Tenth day soil and air temperature

recorded at 2:00 PM

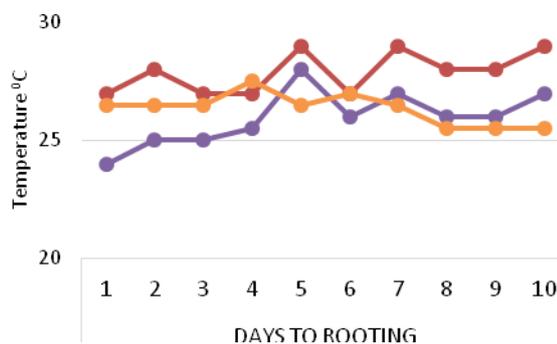


Figure 4c. Tenth day soil and air temperature

recorded at 6:00 PM

Legend:

- w/ BH
- w/o BH
- Air Temp

Relative Humidity inside the chamber

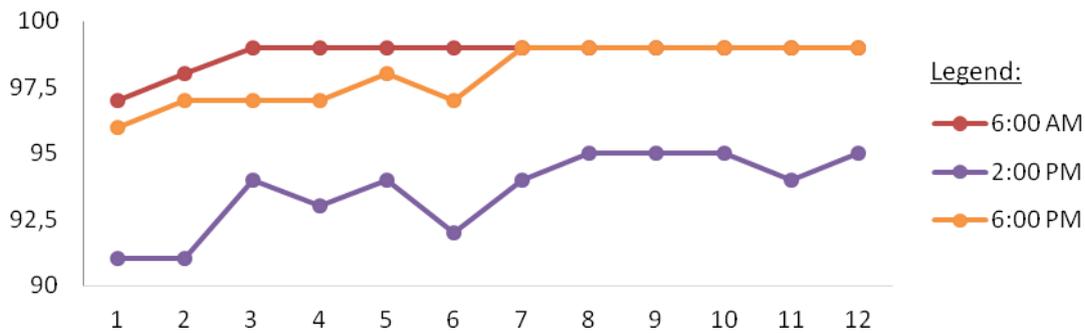


Figure 5. Average weekly Relative humidity inside the chamber throughout the 90 days propagation period

Horticultural Root Characteristics of Regenerated Eugenia Cuttings

Table 1 shows the regeneration characteristics of Eugenia stem cuttings as influenced by bottom heat and type of cuttings. Regardless of the type of cutting, bottom heat application did not significantly influenced all the cutting regeneration parameters evaluated including percent leaf retention, percent rooting, number, length and diameter of primary roots, as well as the fresh and dry weights of the primary roots of Eugenia cuttings. The result indicates that the rooting medium temperature difference of 2 °C between the non-bottom heated (B₁) and the bottom heated (B₂) treatment did not caused significant influence on the regeneration performance of Eugenia stem cuttings. Contrary, regardless of bottom heat treatment, the type of cuttings significantly affected the percentage leaf retention and percentage rooting but had no significant influence on the root characteristics including, number, length, diameter and the fresh and dry weights of roots. Cutting without (T₁) axillary shoot had higher percentage leaf retention than cutting with 1 and 2 axillary shoots retention (T₂ and T₃). The lower leaf retention among cuttings in T₂ and T₃ compared to T₁ could be partly attributed to their bigger leaf surface area that favor higher moisture loss and thus, resulted to unfavorable water balance triggering leaf abscission. High leaf abscission rate associated with high transpiration rate were reported by Grange and Loach (1983), Loach (1988) Ofori et al. (1996) and Aminah et al. (1997). In addition, the lower leaf retention among T₂ and T₃ cuttings could be probably a result of nutrient stress resulting from competition among many leaves in T₂ and T₃ cuttings.

Furthermore, percentage rooting was significantly lower among T₃ cuttings as compared to T₁ and T₂. The lower percentage rooting of T₃ cuttings can be probably attributed to poor nutrient supply reaching the base of the cutting due to unfavorable water balance (high transpiration losses due to big leaf surface area) and to high nutrient demand of the shoot which probably had negative effect on root initiation. Root ignition and root development in cuttings requires adequate supply of nutrients (Haissig, 1986; Davis & Potter, 1989; Cameron, 2005). The effects of poor nutrients supply for rooting process of cuttings was reported by Wu (2011) in *Protea cynaroides* and Sharp (1955) in peach, grape and blueberry stem cuttings which achieved poor rooting percentage.

All cutting regeneration parameters evaluated were all not significantly influenced by the interaction effect of bottom heat treatment and type of cuttings.

Roots Starch and Sugar Content

The root starch and sugar contents of Eugenia cuttings were both not significantly affected the rooting medium temperature manipulation, type of cutting and their interaction effect (Table 2). However, although the difference was not significant, roots of cuttings propagated in medium with bottom heat (B₂) had numerically higher starch content than roots of cuttings rooted in medium without bottom heat treatment (B₁). Furthermore, root of cuttings with one axillary shoot retain (T₂) had slightly higher starch and sugar contents than root of cuttings without axillary shoot (T₁) and with 2 axillary shoot (T₃).

Horticultural Characteristics of Successfully Regenerated Eugenia Stem Cuttings

The successfully regenerated cuttings (Fig. 6) and the horticultural characteristics of the successfully regenerated Eugenia cuttings 30 days after potting are shown in Table 3. Bottom heat application significantly influenced the number and length of axillary shoots, and width of leaves but had no significant influenced on the percent survival, number of days to 50 % shoot formation, number of axillary shoots and length of leaves. After 30 days from planting, cuttings rooted from bottom-heat treated (B₂) rooting medium had longer axillary shoots compared to

cuttings rooted in non-heated medium (B_1). The longer axillary shoots among B_2 cuttings compared to B_1 cuttings could be partly attributed to the slightly earlier shoot development or to the possible beneficial effect of the slightly higher root starch content among B_2 cuttings. It might be that the photosynthates among B_2 cuttings may have been used for shoot development rather than for root development as the roots already have suitable amounts of food/starch.

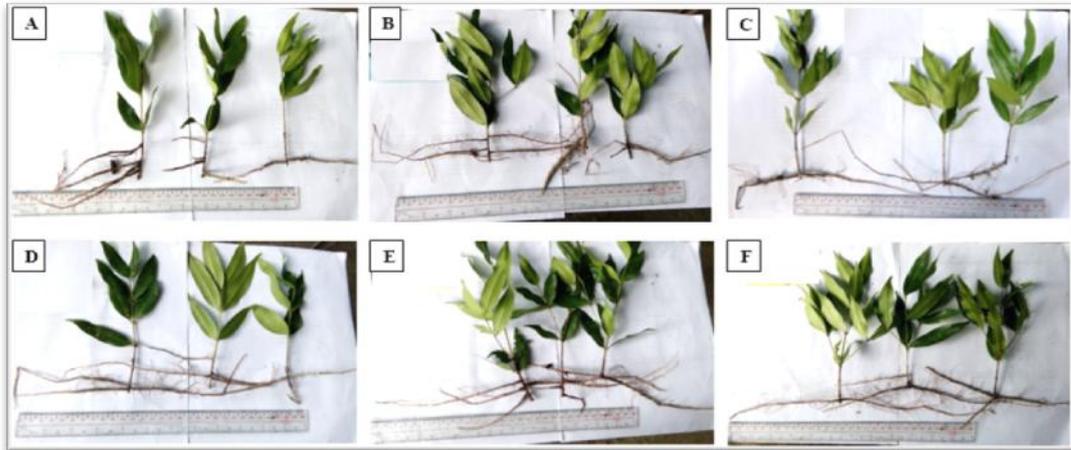


Figure 6. Successfully regenerated *Eugenia* stem cuttings (A- no axillary shoot + BH; B- 1 axillary shoot + BH; C- 2 axillary shoot + BH; D- No axillary shoot without BH; E- 1 axillary shoot + without BH; F- 1 axillary shoot + without BH)

Furthermore, the more and wider leaves among bottom heat-treated (B_2) cuttings relative to non-bottom heat treated (B_1) cuttings could be attributed to the longer shoots produced among (B_2) cuttings.

Regardless of medium temperature treatment, the type of cutting significantly affected the length and width of leaves but had no significant influence on duration of shoot formation, length and number of axillary shoots and number of leaves. Cuttings with 1 (T_2) and 2 axillary shoots (T_3) had wider and longer leaves compared with cuttings without axillary shoot (T_1). The bigger leaves among cuttings with axillary shoots could be a result of better nutrient supply (photosynthates) resulting from higher photosynthetic activity from more leaves. The presence of more leaves in cuttings which influenced the accumulation of photosynthates was reported by Costa (2002) to have strong regulatory effect on the growth of organs such as leaves.

The rooting medium temperature treatment x type of cutting effects on all horticultural characteristics of the planted *Eugenia* cuttings 30 days after planting were all not significant.

Table 1. Leaf retention, percent rooting and root characteristics of *Eugenia* stem cuttings as affected by bottom heat treatment and type of cutting 90 days after staking

Treatment	Percent		Number of primary roots	Length of two longest roots (cm)	Diameter of roots (mm)	Fresh Weight Roots (g)	Dry Weight of Roots (g)
	Leaf Retention	Rooting of cuttings					
Main plot (A): <i>Rooting medium</i>							
B_1 -Without bottom heat	91.33	77.22	2.77	14.02	1.84	1.92	0.13
B_2 - With bottom heat	84.89	67.77	2.75	11.38	1.96	2.20	0.12
Sub-plot (B): <i>Type of cutting</i>							
T_1 - no axillary shoot retained	92.67 a	86.67a	3.00	11.39	1.95	0.71	0.11
T_2 - 1 axillary shoot retained	86.17 b	79.16a	3.10	14.51	1.97	0.68	0.13
T_3 - 2 axillary shoot retained	85.50 b	51.67b	2.25	11.45	1.78	0.65	0.10

Means within rooting medium manipulation and type of cutting column having the same letter do not differ significantly at 5 % level of significance using LSD.

Table 2. Starch and sugar content of roots of Eugenia stem cuttings 90 days after staking

Treatment	Percent (%)	
	Sugar	Sugar
Main plot (A): <i>Rooting medium manipulation</i>		
B ₁ - Without bottom heat	4.05	0.67
B ₂ - With bottom heat	5.82	0.60
Sub-plot (B): <i>Type of Cutting</i>		
T ₁ - no axillary shoots retained	4.27	0.57
T ₂ - 1 axillary shoots retained	5.57	0.81
T ₃ - 2 axillary shoots retained	4.80	0.54

Means within rooting medium manipulation and type of cutting column having the same letter do not differ significantly at 5 % level of significance using LSD

Table 3. Survival and horticultural characteristics of successfully regenerated Eugenia stem cuttings 30 days after planting

Treatment	Percent survival of planted eugenia cuttings	Number Of days to 50% shoot formation	Length of new axillary shoots (cm)	No. of new leaves produce	No. of new axillary shoots	Length of new leaves (cm)	Width of new leaves (cm)
Main plot (A): <i>Rooting medium</i>							
B ₁ -Without bottom heat	85.56	11.89	6.58 b	10.33 b	1.92	5.74	1.44 b
B ₂ - With bottom heat	80.00	10.44	8.37 a	12.41 a	2.31	6.30	1.61 a
Sub-plot (B): <i>Type of cutting</i>							
T ₁ - no axillary shoot retained	86.67	10.00	7.07	9.61	2.08	5.39 b	1.30 b
T ₂ - 1 axillary shoot retained	78.33	11.11	7.31	12.39	2.36	6.11 ab	1.66 a
T ₃ - 2 axillary shoot retained	83.33	12.33	8.05	12.11	1.92	6.56 a	1.62 a

Means within rooting medium manipulation and type of cutting column having the same letter do not differ significantly at 5 % level of significance using LSD

CONCLUSIONS

Regardless of the type of cutting, bottom heat application did not significantly influenced regeneration of Eugenia stem cuttings 90 days after staking but significantly increased the length of shoot, number and width of leaves of successfully regenerated Eugenia cuttings 30 days after planting.

The type of cuttings significantly affected the percentage leaf retention and percentage rooting but did not significantly influenced the rooting characteristics of cutting 90 days after staking. After 30 days of planting the regenerated Eugenia cuttings, there were significant differences on the width and length of leaves.

All cutting regeneration parameters and horticultural characteristics of parameters of planted regenerated cuttings evaluated were all not significantly influenced by the interaction effect of rooting medium temperature manipulation and type of cuttings.

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