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Bottom Heat and Naphthalene Acetic Acid Application Affecting Adventitious Root Formation and Shoot Development of Eugenia (*Eugenia myrtifolia* L.) Stem Cuttings

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Abstract

Eugenia (*Eugenia myrtofolia* L.) is one of the most popular ornamental plant used in landscaping industry but success of rooting of this plant is very low due to its difficulty in propagating by stem cutting. Hence, this study aimed to: (1) determine the influence of different levels of Naphthalene Acetic Acid (NAA) on the regeneration of Eugenia stem cuttings; (2) determine the influence of bottom heat application on the regeneration of Eugenia stem cuttings; and (3) evaluate the interaction effect of bottom heat treatment and the different levels of concentration of Naphthalene Acetic Acid (NAA) 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 5 split-plot arranged in RCBD with three replications. The Main-plot was rooting medium temperature manipulation (B1- bottom heat and B2- without bottom heat) and the levels of NAA (T1- o ppm, T2- 500 ppm, T3- 1000 ppm, T4- 1500 ppm, T5- 2,000 ppm, and T6- 2,400 ppm) was the sub-plot. Application of bottom heat in the rooting medium significantly increased percent rooting and number of primary roots. It also increased length of shoots and number and width of leaves regenerated Eugenia stem cuttings 30 days after potting. Moreover, regardless of bottom heat treatment, application of Naphthalene Acetic Acid significantly enhanced leaf retention; increased rooting percentage; number, diameter and length of roots; fresh and dry weight of roots; and roots starch and sugar contents. It also significantly improved the length and number of axillary shoots and number, length and width of leaves of the regenerated cuttings 30 days after potting. NAA concentration of 2,000 ppm was the optimum concentration for successful regeneration of Eugenia stem cuttings. There was no significant interaction effects between bottom heat and Naphthalene Acetic Acid application on the regeneration of Eugenia stem cuttings and the horticultural characteristics of the regenerated cuttings after potting.

Keywords: *Eugenia myrtifolia,* stem cuttings, bottom heat, Naphthalene Acetic Acid, Regeneration

I. INTRODUCTION

Eugenia (*Eugenia myrtifolia* L.*)* is a worldwide plant considered as versatile evergreen shrub or tree. It is known for their attractive glossy foliage especially when its branches were regularly trimmed (Stearn, 2014). Nowadays, it is a highly in demand ornamental plant by many landscapers and household ornamental enthusiasts not only because of its attractive bushy type foliage but because its foliage is easily transformed into different shapes or made into topiaries.

Currently, Eugenia is commonly propagated by stem cuttings because of its irregular seed production (Toussaint *et al*.,1991). Percent regeneration of Eugenia stem cuttings is generally low and often cuttings require longer time to form adventitious roots compared to other plant species (McMullen, 2011). Possible strategies to improve rooting of hard-to-root species such as Eugenia include rooting medium temperature manipulation and root inducers application (Hartmann, 1997). In the case of Eugenia however, only very few studies had been conducted to evaluate the rooting response of Eugenia cuttings to rooting temperature manipulation and root inducer application. Results of the experiment conducted by Lebrun (1998) showed that Eugenia cuttings rooted in non-heated substrate (average of 16 ^{0}C) failed to root while those in heated substrate (average of 22±0.5 ^{0}C) gave 75% rooting on the best substrate. In other plant species, Mitchell (2012) reported that increasing the medium temperature more than 30 ^{0}C reduced the rooting percentage of *Pinus patula* and *Pinus ellioti* x *Pinus caribaea* stem cuttings. Contrary, Chong (2003) revealed that rooting performance of six evergreen taxa (Hick's yew, Hetz juniper, Savin juniper, Ramlosa juniper, Tamarix juniper, and Arborvitea) was significantly higher at bottom heated rooting medium at 12±2^{0}C than that at 21±2^{0}C due to rotting.

The influence of different levels of NAA application on regeneration of Eugenia evaluated

by Lebrun (1998) showed that application of 500 ppm NAA resulted to the highest percent rooting. However, for other species, Beyl and Trigiano (2015) reported that the effectiveness of different NAA concentrations in enhancing rooting of cuttings varies from species to species. Among the NAA concentrations reported to good rooting response were 250 ppm for *Acacia auriculiformis* (Hu and Shen, 1996); 100 ppm for *Dracontomelon dao (*Aquino, 2007) and 2,000 ppm for Lauan (Manipula and Marquita, 1998). Considering the economic importance of Eugenia and the problem associated to its mass propagation through stem cuttings, there is a need to generate more information that could improve its cutting propagation efficiency, hence this study. It aimed to determine the influence of bottom heat application on the regeneration of Eugenia stem cuttings; determine the influence of different levels of Naphthalene Acetic Acid (NAA) on the regeneration of Eugenia stem cuttings; and evaluate the interaction effect of bottom heat treatment and the different levels of concentration of Naphthalene Acetic Acid (NAA) on the regeneration of Eugenia stem cuttings

II. METHODOLOGY

Experimental Design and Layout

The experiment was laid out following a 2 X 5 split-plot in RCBD with three replications having ten (10) sample cuttings per treatment per replicate. The following treatments were evaluated, as follows:

Main-plot *(Soil Temperature Manipulation)*
B_1- no bottom heat applied
B_2- bottom heat applied

Sub-plot *(NAA concentration)*
T_1- 0 ppm, control
T_2 - 500 ppm
T_3 -1,000 ppm
T_4- 1,500 ppm
T_5- 2,000 ppm
T_6- 2,400 ppm

Construction of Propagation Chamber and Rooting Medium

Propagation box 1.5 m wide, 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 at the same time serve as insulator (Fig. 1). Mixture of alluvial soil and finely chopped coconut husk were 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, it was first sterilized by heating over fire for 4 hours. The rooting box was half-filled with the sterilized rooting medium to a medium thickness of about 10 cm.

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

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 box.

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.

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 the 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, ten (10) cm long cuttings were prepared, bundled, and labeled according to the assigned treatments.

Preparation of Naphthalene Acetic Acid Treatment of Cuttings

Hormex (Ramgo) was used as the rooting hormone. The NAA solutions having different concentration (500 ppm, 1,000 ppm, 1,500 ppm, 2,000 ppm and 2,400 ppm) were prepared by diluting pure Hormex solution (2,400 ppm) with clean tap water using the formula $C_1 V_1 = C_2 V_2$ were; C1- pure Hormex NAA concentration, C2- desired NAA concentration, V1- volume of stock solution, and V2- target volume of NAA solution to be prepared.

The base of the cuttings were uniformly dipped into the solution having different NAA concentration for 15 minutes.

Staking of Cutting

The rooting medium was moistened first with clean tap water before inserting the individual cutting. 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 - rotting medium contact. These were sprayed with fungicide solution (Dithane M-45) at 1tbsp/gallon of water right after staking and at biweekly interval thereafter.

Care and Maintenance

The RH was regularly checked 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 a 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.

Planting of Successfully regenerated Cuttings

Thirty successfully rooted cuttings (ten per replication) were planted in plastic bags containing 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.

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 ^{O} 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

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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.

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.

III. 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 Fig 3a, 3b, and 3c. The temperature of the rooting medium applied with bottom heat was about 6.3 ^{0}C higher than that of the rooting medium without bottom heat treatment and about 5.23 ^{0}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 32.7, 26.4 and 27.47 ^{0}C, respectively. The difference temperature between bottom-heated rooting medium and air temperature are almost thrice higher than the temperature (2 ^{0}C) evaluated by McGroaty (2015) in promoting adventitious root initiation in cuttings.

However, no difference of temperature was achieved between non-bottom heated medium and air temperature. Furthermore, the average temperature of bottom-heated medium was in the optimum level (25 ^{0}C to 32 ^{0}C) as evaluated by Dykeman (1976) and Kester (1970) in propagating warm-climate species or even higher to the range (25 ^{0}C to 28 ^{0}C) as reported by Mitchell (2012) in increasing the root weight of *Pinus patula* stem cuttings. Thus, the rooting medium temperature from bottom heat was conducive in stimulating the root initiation of Eugenia cuttings.

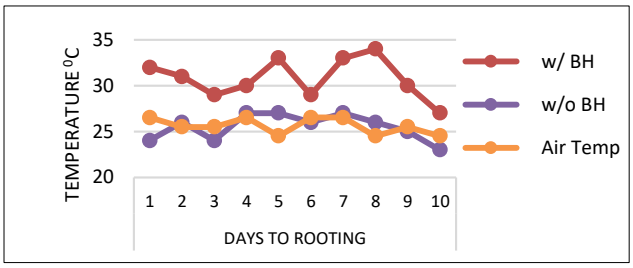


Figure 3a. Ten days soil and air recorded at 6:00 AM

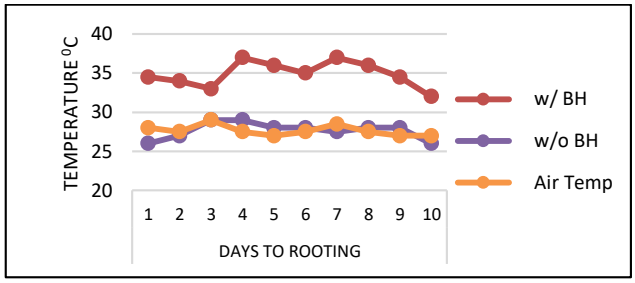


Figure 3b. Ten days soil and air recorded at 2:00 PM

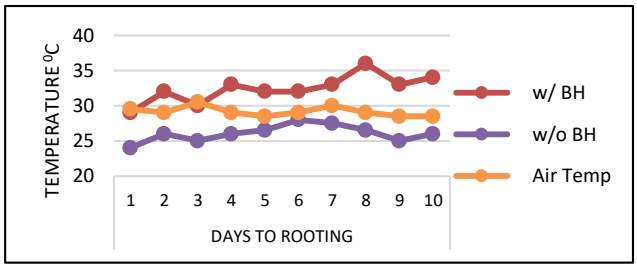
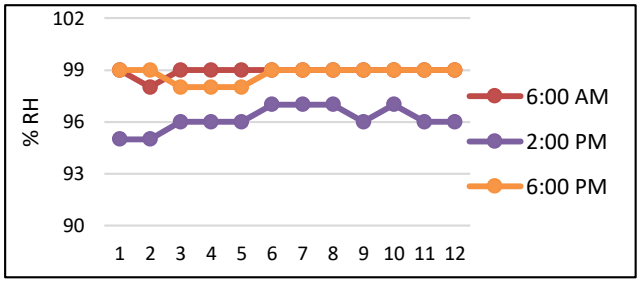


Figure 3c. Ten days soil and air temperatures recorded at 6:00 PM



Weeks days of rooting period

Figure 4. Average weekly percent RH inside the propagation chamber throughout the 90 days propagation period

Likewise, the average relative humidity inside the propagation chamber at 6 am was 98.92%, 96.17 at 2 pm and 98.75% at 6 pm. The average daily RH during the 12- week propagation period was 97.94% indicating that a humid condition conducive to rooting was

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

TREATMENTS	PERCENT		NUMBER OF PRIMARY ROOTS	LENGTH OF TWO LONGEST ROOTS (cm)	DIAMETER OF ROOTS (mm)	FRESH WEIGHT ROOTS	DRY WEIGHT OF ROOTS (g)
	LEAF RETENTION	ROOTING OF CUTTINGS					
Main plot (A): *Rooting medium manipulation*							
B_1-Without bottom heat	74.39	56.94b	1.96b	8.66	1.03	0.62	0.13
B_2- With bottom heat	72.17	69.17a	2.38a	9.92	1.30	0.76	0.14
Sub-plot (B): *Levels of NAA*							
T_1 – 0 ppm, control	61.00b	37.50b	1.10b	6.94b	0.89b	0.34 c	0.11 b
T_2 - 500 ppm	65.83ab	63.33a	2.22a	8.80ab	1.05ab	0.72 ab	0.13 b
T_3 - 1,000 ppm	78.00ab	59.17a	2.38a	8.80ab	1.43a	0.78 ab	0.28 ab
T_4 - 1,500 ppm	76.83ab	68.33a	2.18a	9.69ab	1.18ab	0.74 ab	0.13 ab
T_5 - 2,000 ppm	83.33a	74.17a	2.48a	10.21a	1.36a	0.91 a	0.20 a
T_6 - 2,400 ppm	74.17ab	75.83a	2.65a	11.29a	1.23ab	0.67 b	0.12 ab
CV (%) (A)	8.86	11.29	7.98	21.81	33.68	21.35	24.81
(B)	15.97	15.38	25.47	16.65	20.3	39.45	29.85

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.

maintained inside the chamber during the entire propagation period (Fig. 4). This was even higher than 85 % which was considered optimum RH for rooting stem cuttings (Hartmann *et al*., 1997).

Horticultural Root Characteristics of Regenerated Eugenia Cuttings

Table 1 shows the regeneration characteristics of Eugenia as influenced by bottom heat and varying levels of NAA. Regardless of the levels of NAA, bottom heat application (B_2) significantly influenced rooting percentage and number of primary roots but had no significant influenced on other cutting regeneration parameters of Eugenia stem cuttings. The result indicated that the rooting medium temperature difference of 6.05 ^{0}C between the non-bottom heated (26.4 ^{0}C) and the bottom-heated treatment (32.7 ^{0}C) had significantly enhanced rooting percentage and number of primary roots of Eugenia stem cuttings. The improvement in percentage rooting in Eugenia cuttings with increasing rooting medium temperature was in agreement with the findings of Lebrun (1998) in Eugenia cuttings and Whalley *et al*. (1977) in rhododendron cuttings. Improve rooting of cuttings resulting from higher rooting medium temperature was attributed to greater metabolic activity of the cutting base which induces root initiation while retarding shoot growth (Wildon, 1929, McGroaty, 2015; Richards, 2015; Lopez & Runkle, 2005).

Regardless of bottom heat treatment, the different levels of NAA had significantly influenced on percent leaf retention, percent rooting, number of primary roots, length and diameter of two longest roots, and root fresh and dry weights. Cutting applied with 2,000 ppm NAA had significantly higher leaf retention than the control but had comparable leaf retention with cuttings treated with the other four NAA levels. Likewise, application of five levels of NAA (500 ppm, 1,000 ppm, 1,500 ppm, 2,000 ppm and 2,400 ppm) significantly increased percent rooting and number of primary roots relative to the non-treated cuttings. Furthermore, cuttings treated with 2,000 and 2.400 ppm NAA had significantly longer and broader roots than the control although their root lengths and diameter were just comparable with those treated with 500 to 1,500 ppm NAA. In terms of root weight, cuttings treated with 2,000 ppm NAA had higher fresh and dry weight than the non-NAA treated cuttings. Root weight among cuttings treated with the other four levels of NAA were just comparable. In terms of rooting percentage and root quality of Eugenia cuttings, 2,000 ppm NAA appeared to be the optimum level which was higher than the NAA level of 500 ppm reported by Lebrun (1998) to be the effective concentration for better rooting of Eugenia cuttings. In *Hermarthria compressa,* Yan (2014)

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found that the application of 2,000 ppm of NAA significantly increased rooting percentage, with more and heavier adventitious roots. Manipula and Marquita (1998) reported 87% survival in lauan cuttings treated with 2,000 ppm NAA.

All cutting regeneration parameters evaluated were not significantly influenced by the interaction effect of bottom heat treatment and NAA concentrations.

Root Starch and Sugar Contents

The root starch and sugar contents of regenerated Eugenia stem cuttings were not significantly affected by bottom heat application but was significantly influenced by NAA levels (Table 2).

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

TREATMENT	PERCENT (%)	
	STARCH	SUGAR
Main plot (A): *Rooting medium manipulation*		
B_1 - Without BH	4.47	0.69
B_2 - With BH	4.63	0.69
Sub-plot (B): *Levels of NAA*		
T_1 – 0 ppm, control	2.88 b	0.36 c
T_2 - 500 ppm	4.65 a	0.72 b
T_3 - 1,000 ppm	4.53 ab	0.72 b
T_4 - 1,500 ppm	5.09 a	0.57 bc
T_5 - 2,000 ppm	5.75 a	1.05 a
T_6 - 2,400 ppm	4.37 ab	0.73 b
% CV (A)	21.37	15.72
(B)	4.55	24.32

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.

Regardless of bottom heat application, increasing the levels of NAA application from 500 ppm to 2,000 ppm correspondingly increased the root sugar and starch content with an optimum level of concentration at 2,000 ppm NAA. The influenced of NAA in increasing the starch and sugar content of Eugenia could probably attributed by the good quality of roots produced in 2,000 ppm (Table 1). The higher production of starch content in root was reported by Wu (2011), Haissig (1986), Veierskov (1988), Husen and Pal (2007) that NAA have initiates higher metabolic activity for food reserves resulted to an increased accumulation of energy source (starch) at the base of cutting.

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 LEAVES (cm)	WIDTH OF LEAVES (cm)
Main plot (A): *Rooting medium manipulation*							
B_1 - Without bottom heat	79.44	8.44	7.33b	11.72b	2.30	5.11	1.78 b
B_2 - With bottom heat	78.33	8.61	8.23a	12.82a	2.26	5.63	1.86 a
Sub-plot (B): *Levels of NAA*							
T_1 – 0 ppm, control	56.67 d	9.66	6.17c	8.42b	1.64c	4.29b	1.59b
T_2 - 500 ppm	73.33 c	8.83	7.50b	13.10a	2.03bc	5.16ab	1.83ab
T_3 - 1,000 ppm	76.67 bc	8.17	8.05ab	12.48a	2.42ab	5.43ab	1.85a
T_4 - 1,500 ppm	85.00 abc	8.00	8.13ab	12.72a	2.18bc	5.65a	1.86a
T_5 - 2,000 ppm	93.33 a	7.67	8.65a	13.93a	2.92a	6.07a	1.99a
T_6 - 2,400 ppm	88.33 ab	8.33	8.20ab	12.98a	2.58ab	5.63a	1.83ab
% CV (A)	5.33	21.14	5.60	6.15	18.74	13.19	14.07
(B)	8.05	19.29	7.93	9.77	16.21	12.26	7.67

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.

Horticultural Characteristics of Successfully Regenerated Cuttings 30 Days after Planting

The successfully regenerated cuttings and the horticultural characteristics of the successfully regenerated Eugenia cuttings 30 days after planting are shown in Table 3. The horticultural characteristics of the successfully regenerated among NAA treated cuttings could be attributed to better root quality (Table 1) as well as better shoot quality (Table 3). Furthermore, cuttings treated with 1,000 ppm to 2,400 ppm NAA had significantly more, longer axillary shoots, with more and bigger leaves compared to the non-treated control, and those treated with 500 ppm NAA. Again, the improve shoot development of NAA treated cuttings compared to the non-NAA treated once could be attributed to better root quality and higher starch and sugar content of the NAA treated cuttings. The result showed that optimum shoot development of Eugenia stem cuttings was achieved by treating cuttings with 2,000 ppm NAA. The improved shoot and wider leaves produced from 2,000 ppm NAA was reported by Khudhur and Omer (2015) on *Dalbergia sissoo* and Nikita (2015) on black pepper stem cuttings that application NAA had mobilizes more food reserve (starch) as a sink during root development, which increases the translocation of growth substance, resulted to longer shoot and more and wider leaves.

IV. CONCLUSION

Therefore, bottom heat application increased percent rooting, and number of primary roots of regenerated Eugenia stem cuttings and increased the length of shoots and number of leaves of successfully regenerated Eugenia cuttings thirty days after potting. NAA application at the rate of 2,000 ppm significantly increased the rooting percentage, percent leaf retention, number, size and weight of roots as well as the starch and sugar content of roots of Eugenia cuttings. The same NAA application level significantly increased the number and length of axillary shoots, number and size of leaves of Eugenia cuttings after planting. There was no significant interaction effects between bottom heat and Naphthalene Acetic Acid application on the regeneration of Eugenia stem cuttings and the horticultural characteristics of the planted regenerated cuttings.

For propagating Eugenia by stem cutting, treating by dipping in 2,000 ppm NAA for 15 minutes is recommended. Further investigation on the regeneration response Eugenia stem cutting to bottom heat must be carried out to verify the present results.

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