ROOTING RESPONSE OF MAGIC FRUIT (*Synsepalum Dulcificum* Daniell) STEM CUTTINGS TO APPLICATION OF INDOLE-3 BUTYRIC ACID

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---**ABSTRACT**---
Magic fruit can be asexually propagated through hardwood and softwood cuttings using different root-initiating substances. Due to its high economic value in the medical and food industry, propagation study of this species is necessary to supply large number of planting materials. This study examined the rooting response of different types of cuttings (softwood and hardwood) magic fruit cuttings to application of different levels of indole-3 butyric acid (0, 200, 400, 600, 800 and 1000 ppm of IBA solution). Results of the study showed that increasing rates of IBA from 200 to 800 ppm promoted higher percentage rooting on both types of cuttings, but 800 ppm of IBA produced the highest percentage of 81.67%. While softwood cuttings responded favorably to application of IBA in terms on the number of days to emergence of new leaves and number of roots developed in magic fruit cuttings. Development of new leaves was shortened to 18-25 days, while in hardwood cuttings it was delayed to 30.10 days after planting. Based on the result, the optimum level of IBA was 800 ppm and hence recommended for rooting of magic fruit through stem cuttings.
KEYWORDS: *hardwood cutting, rooting, softwood cutting*---

INTRODUCTION

Magic fruit (*Synsepalum dulcificum* Daniell) is an evergreen shrub, whichbelongs to Sapotacea family originating from West Africa (Keay, 1992). As reported by Opeke (1984), this species is naturally grown in its native habitatin the wild area of rain forest, and it can survive even in the full sunshine and drought. When this crop is planted in container it can grow up from 1.2 to 1.8 meters in height while in its natural habitat in can reach 3 to 4.5 meters. Ogunsola and Ilori (2008), cited that the small ellipsoid berries are just about 2 to 3 cm long, surrounded by edible pulps that are bright red when ripe.

Magic fruit belongs in the category of promising products, in fact other countries already consumed the product as an alternative pill just to avoid bitter and sour flavors. In Japan, magic fruit is commercialized as a fruit, powder and tablet forms. Whereas, in Tokyo, they served dessert that have less calories and it does not exceed to 100 calories per serving. Said products are also popularly consumed by dieters and diabetics patients in Japan (calorielab.com/ news/2005). As mentioned by Peregrin (2009), patients with chronic disease who developed unpleasant taste during their treatments had utilized this fruit to make food taste better. While diabetic patients take this as an alternative sugar as it is considered safe and have no side effects (Kant, 2005 and Levin, 2012). In the Philippines, Dr. Angeles produces sweet wine with a distinct flavor from the magic fruit berries. He markets his wine under the D'Estan brand name. It has a very distinct taste, and this helps distinguish it from other wines in the local market (https://www.zacsarian.com /Agriculture News, Vol. XIX September 2015). It has been proven by the Food and Drugs Administration (FDA) that miraculin compounds present in the fruit were safe and effective as sugar substitute. This claimed was further supported by Dr. Linda Barthoshuk based on her experiment conducted for Smell and Taste in University of Florida Center that consumption of this fruit has no negative effect which werehazardous to human health. Hence, it has been commercialized as substitute natural food sweetener for it has been certified and proven safe and that little amount was utilized to make the food taste sweet as compared to the excessive use of food additives (Bartoshuk et al., 1974).

The interesting fact about this fruit is the taste modifying properties of the fleshy pulp that can alter all sour foods into sweet when consumed (Yong, 2011). Such effect would last until such time that the miraculin was disband and eliminated by saliva (Rehm and Espig, 1991).

Numerous studies have revealed that the sweetening effect of its flesh pulp is due to the presence of miraculin, which is known as a glycoprotein that binds to receptors of the taste buds and alter their function (Metcalfe and Chalk, 1972).

At present, magic fruit has been considered as an important source of medicinal plant. As mentioned by Peregrin (2009), patients with chronic disease who developed unpleasant taste during their treatments had utilized this fruit to make food taste better. Most et al. (1979) also reported that this magic fruit was consumed for centuries by West African people to sweeten their sour foods and drinks. While diabetic patients take this as an alternative sugar as it is considered safe and have no side effects (Kant, 2005 and Levin, 2012). It has been proven by the Food and Drugs Administration (FDA) that miraculin compounds present in the fruit were safe and effective as sugar substitute. This claimed was further supported by Dr.Linda Barthoshuk based on her experiment conducted for Smell and Taste in University of Florida Center that consumption of this fruit has no negative effect which were hazardous to human health. Hence, it has been commercialized as substitute natural food sweetener for it has been certified and proven safe and that little amount were utilized to make the food taste sweet as compared to the excessive use of food additives (Bartoshuk et al., 1974).

Due to commercial potential of miraculin because of its nutritional and medicinal values, there is a need to mass produce and conduct further research to develop its possible uses. However, commercial production of magic fruit has been a constraint as it was reported by Joyner (2006), that regeneration of this species by cuttings is difficult. Though, numerous studies showed that regeneration of woody tree species has been proven effective when it is assisted by Indole-3-Butyric Acid (IBA) (Kesari et al., as cited by Xingwei, 2013). It was reported that an increasing level of application of IBA resulted to increased rooting percentage (Kesari et al., 2009; Lemay et al., 2009). Hence, technique in propagating magic fruit plant through cuttings needs to be developed to generate numerous planting materials. This study aimed to determine the effect of different concentrations of IBA hormone on softwood and hardwood stem cuttings in promoting adventitious root in magic fruit.

MATERIALS AND METHODS

Study Site

This study was carried out at the experimental area of University of Southern Mindanao Research and Development Center, Kabacan, North Cotabato, Philippines (7°7′12″N, 124°49′12″E) from November 2018 to March 2019. The area has an elevation of 21 m.

Planting Material

The smaller leaf-version of Synsepalum species was used in the study. Cuttings were collected from a healthy, fruit bearing and disease-free magic fruit plant from the orchard of Dr. Ariston Calvo at Osias, Kabacan, North Cotabato.

Experimental Set-up

Magic Fruit Cutting Experiment

The type of stem cuttings of magic fruit such as softwood and hardwood was investigated. Softwood stems of magic fruits were selected and carefully cut into 5 - 10 cm length with 2 to 3 nodes and 2 to 5 leaves on the upper part. For hardwood cuttings, healthy stems were selected with one pair of leaves and 1-3 nodes and cut into sections of 5 - 10 cm in length. Using a sharp budding knife, the leaves were cut by half to reduce transpiration. These cuttings were transported and handled properly from the source to the experimental area.

The soil rooting media used were topsoil and the coconut coir dust in 1:1 ratio. The prepared rooting media was placed in a 4” x 6” transparent plastic bag at ¾ full followed by the application of fungicide to destroy fungi and other microorganisms present in the potting media. Cuttings were planted by inserting the basal portion of the stem cuttings for at least 2-3 cm depth for both softwood and hardwood cuttings. All samples were then placed in a partly shaded area in an enclosed system propagation that serves as a modified vacuum condition. Plants were arranged accordingly in a Completely Randomized Design (CRD) in factorial arrangement with 12 treatment combinations having three replications with ten sample cuttings each treatment.

Rooting Experiment

The performances of softwood and hardwood cuttings treated with IBA were investigated. The basal portions of both softwood and hardwood cuttings were quickly dipped for at least 5 minutes separately into a previously prepared and predetermined IBA solutions (0, 200, 400, 600, 800 and 1000 ppm) as described by Hartman (2002) using plastic container for dipping of cuttings of at least 1 cm depth based on the specific treatments. To prepare a 100 ml solution with a concentration of 200 ppm, 2 ml of the stock solution was added to 98 ml water. Each solution was kept separately in a bottle. Treated cuttings were then planted in rooting media and maintained in a modified vacuum condition for 90 days. Plants were arranged in a Completely Randomized Design (CRD) in factorial arrangement. Type of stem cuttings of magic fruit such as softwood and hardwood served as Factor A, while the varying levels of IBA served as Factor B. There were 12 treatment combinations having threereplications with 10 sample cuttings each treatment.

Plant Growth Parameters

Growth parameters were assessed at 90 days after planting to determine root formation on stem cuttings of magic fruit. Days to emergence of new leaves were taken by counting the number of days to emergence of new leaves that developed in the cuttings. The number of leaves was determined by counting the number of newly developed leaves per plant sample. To assess the root formation, cuttings were uprooted and washed with running water. The number of roots was counted per plant. The rooting percentage was computed using the formula

$$\text{Rooting percentage} = \frac{\text{Number of rooted cuttings}}{\text{Total number of cuttings}} \times 100$$

The percentage survival (%) was computed using the formula

$$\text{Percentage survival} = \frac{\text{Number of survived cuttings}}{\text{Total number of cuttings}} \times 100$$

Statistical Analysis

Parameters gathered from the magic fruit cutting experiment were analyzed using the Statistical Tools for Agricultural Research (STAR). Statistical significance of differences between mean values was determined using Tukeys (HSD) and LSD *post hoc t*ests with a level of significance of 0.05.

RESULTS AND DISCUSSION

Tables 1 to 5 respectively present the number of days to emergence of new leaves, number of leaves, number of roots, rooting percentage, and survival percentage of magic fruit cuttings 90 days after planting (DAP). Significant interaction effect between two factors was found only in the number of days to emergence of new leaves while for the type of cutting, significant differences was found in the number of leaves. With respect to varying levels of IBA, significant differences were found in the number of roots developed per cutting and percentage rooting.

Days to Emergence of New Leaves

Statistical analysis revealed a highly significant interaction effect between types of cuttings and levels of IBA at 90 DAP (Table 1). Results clearly indicated that softwood cuttings without IBA treatments significantly developed leaves earlier (18.53 days) than other treatments but comparable to those cuttings treated with 200, 400 and 1000 ppm. It was further noted that softwood cuttings treated with 600 ppm, showed delayed appearance of new leaves to 29.74 days after planting. While in hardwood cuttings, treatments with high level of IBA (1000 ppm) initiated early emergence of new leaves (26.78 days) and the lowest concentration of 200 ppm prolonged appearance of new leaves up to 30 days after planting, as evident from the data presented.

Significant differences were also observed on the types of cuttings, softwood cuttings developed new leaves earlier with a mean of 24.04 than hardwood cuttings with an average number of 30.10 days.

This implies that softwood cuttings were better than hardwood cuttings in terms on the number of days to emergence of new leaves, as days to appearance of new leaves was shortened by 8 days, while hardwood cuttings took time (26.78 days) in producing leaves. The result can be attributed to the presence of higher auxin in the terminal section of the stems and active buds were also present on cuttings, which enhanced early development of new leaves. Similar findings was noted in the study of Agbo and Obi (2007) on *Gongronema latifolia* Benth, wherein softwood cuttings had significant(p=0.05) lower number of days to opening of apical bud and shoot development coupled with its early development of opposite leaves by the second week after planting.

Number of Newly Developed Leaves

Statistical analysis revealed a highly significant effect on the number of leaves of magic fruit cuttings at 90 DAP. The types of cuttings significantly influenced this parameter (Table 2). Hardwood cuttings regardless of IBA treatments developed more leaves when compared to softwood cuttings with a mean of 4.99 leaves, which differed significantly with hardwood cuttings with an average number of 7.12 leaves. The same result was obtained by Tadele et al.(2018) wherein highest leaf number were recorded from hardwood rose cuttings (*Rosa canina inermis*). This implies that hardwood magic fruit cuttings tended to develop more leaves than softwood cuttings at any levels of IBA treatments.

The different levels of IBA did not significantly affect the number of leaves developed in magic fruit stem cuttings. This conforms to the findings of Magdalera (1980), who observed that the number of leaves of breadfruit cuttings was not significantly increased by the application of 0 to 500 ppm ANAA. However, Fabia, as cited by Pangilamen (2003) reported that in calamansi cuttings, the application of 200 ppm of IBA promoted the development of more leaves.

As observed, the development of more leaves to hardwood cuttings have affected the initiation of roots. Delayed root formation has been noticed in cuttings that showed early development of leaves. Most probably, these leaves may acted as competing organs for the use of stored carbohydrates hence, slowed down growth or initiation of roots.

Moreover, hardwood cuttings developed more leaves and take time to developroots when compared to softwood cuttings, maybe due to the fact that hardwood cuttings contain stored foods such as hydrocarbons, nucleic acids, proteins and natural hormones such as IAA and or cytokinins that can be usedfor the development of leaves (Hambrick et al., 1991). The stored foods is an important feature for the growth and development capacity of stem cutting which are more in quantity in hardwood than in softwood cuttings that enable the hardwood cuttings to grow and develop more leaves than other types of cuttings. The presence of more leaves on hardwood cuttings exerts a strong stimulating influence on root initiation (Reuveni and Raviv, as cited by Cordon, 2006).

Number of Roots

The average number of roots of softwood and hardwood magic fruit cuttings was significantly influenced by the application of varying levels of IBA asshown in Table 3. Cuttings without the application of IBA had the lowest number of roots (4.99) which was twice the number of roots developed in cuttings treated with the highest level of IBA (1000 ppm) with the highest mean of 10.75 roots comparable to cuttings treated with 600 ppm of IBA. This observation confirms the report of Pangilamen (20) who stated that varying levels of ANAA has a significant effect on the number of roots developed in pomelo cuttings. The number of primary roots increases as rates of ANAA increased from 250 to 1000 ppm.

The result implies that application of IBA at a high concentration of 1000 ppm regardless of types of cuttings is better in promoting root development in magic fruit cuttings, which could also lead to higher survival.

On the other hand, types of cuttings did not significantly affect the number of roots in magic fruit. Both types of cuttings revealed a favorable response to higher IBA treatments. The current findings agree with Beldia (2003) who reported no significant difference on the roots developed between semi-harwood and hardwood lanzones cuttings soaked in 500-1000 ppm ANAA.

Erratic result was observed among the levels of IBA regardless of types of cuttings as number of roots developed was higher at 600 ppm (11.38) but decreased (7.14) when IBA treatment was increased to 800 ppm and increased (10.75) at 1000 ppm. Inconsistent result might be due to physiological condition of the cuttings and the unfavorable environmental conditions during critical rooting period. Hartmannm et al. (1997) reminded that auxin treatment is not absolute guarantee for root formation of cuttings. They further emphasized that physiological as well as environmental condition of the stocks play and important role in the process. High temperatures,greater than 32^{o}C, hasten evapo-transpiration rate, slow down cell processes, cause injury to cells and eventually impede rooting.

Percentage Rooting

The percentage rooting 90 days after planting is shown in Table 4. Statistical analysis revealed no significant interaction effect between two factors on rooting percentage of magic fruit cuttings with means ranging from 30.0 to 90.0%. Type of cuttings did not significantly affect the rooting percentage of magic fruit. However, varying levels of IBA significantly influenced this parameter. Increasing rates of IBA from 200 to 800 ppm promoted higher percentage rooting in softwood and hardwood cuttings. Cuttings treated with 800 ppm of IBA gave the highest percentage of 81.67%, which is 50% higher than control treatment with 31.67%, but comparable to cuttings treated with 400 and 600 ppm with similar means of 68.33%.

Previous studies revealed that the percentage rooting increased with higher IBA concentrations. The same pattern was noted in the study of Chen et al. (2012); Duke and Ducellier (1993) where softwood miracle fruit cuttings treated with 800 mg/L of IBA gave higher rooting percentage.

The rooting percentage of softwood cutting was slightly higher by 9.45% than hardwood cuttings. This confirmed with the report of Coronel (1983) thatyounger cuttings treated with root promoting chemicals and rooting them under mist have been more successful than mature cuttings.

On the other hand, cuttings treated with 1000 ppm of IBA had significantly lower percentage of rooting (41.67%) comparable to untreated (31.67%) and reduced percentage rooting particularly the softwood cuttings. This only confirms that treating cuttings with auxin may not always ensure formation of adventitious roots. Hartmann and Kester (1975) stated that a concentrationjust below the toxic point is considered as the most efficient and favorable for root formation. They believed that plant produces root-promoting and root- inhibiting substances that are thought to be involved in the root formation of cuttings. They also mentioned that difficult-to-root cuttings have high levels of root-inhibiting substances. As reported by the scientists in early 1950’s, endogenous chemical inhibitors retarded rooting in selected plant species and this was particularly observed in grapes cultivar (Yeboah 2015). In a study conducted by Cuir et al. (1993), a cinnamic acid derivative that inhibits rooting was observed in a difficult-to-root hardwood cuttings of wax flower (*Chamaellaucim uncinatum*), while in easy-to-root softwood cuttings no detectable levels of this phenolic compound was found. Yeboah (2015)also stated that cuttings of difficult-to-root mature eucalyptus, chestnut and Dahlia cultivars also showed higher rooting inhibitors than easy-to-root cuttings. This simply means that high concentration of IBA could be potential rooting inhibitor.

Other factors such as physiological and environmental conditions should also be taken into consideration. Hartmann et al. (1997) cautioned that controlled temperature is necessary and is very important factor in the rooting of cuttings;if possible, 23 to 27^{0}C at the base of cuttings and 30 to 32^{0}C to ambient should be maintained during rooting, as it will give satisfactory results in many plants.

During the conduct of this study, the microclimatic condition in Kabacan was constantly hot and oppressive because of the El Niño condition, which started to unfold during the last quarter of 2018 until the first quarter of 2019. From January 2018 to March 2019, an extreme temperature of 37.4^{0}C was observed in Cotabato City stations monitored by the weather and climate authority (*source:DOST-PAGASA El Niño Advisory no.2, March 2019*. Said temperature was too high particularly during critical rooting period of cuttings. Maybe some cuttings, particularly those cuttings that were applied with low levels of IBA may not be able to tolerate unfavorable conditions of high temperature and it disrupts the rooting formation of cuttings.

The result implies that the optimum level of IBA for rooting of magic fruit regardless of types of cuttings is 800 ppm with favorable environmental conditions. Beyond which, development of adventitious roots may be prevented.

Percentage Survival

Table 5 presents the percentage survival of magic fruit cuttings applied with different levels of IBA at 90 days after planting. In this parameter, cuttingswere considered as survived when the leaves, shoots, buds or even roots are present in the cuttings.

Statistical analysis revealed no significant interaction effect between two typesof cuttings and levels of IBA on the percentage survival of magic fruit stem cuttings. Similar trend of results was observed regardless of types of cuttings and the levels of IBA treatment. The percentage survival ranged from 77 to 100%. The result

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implies that exogenous application of auxin may not be necessary to attain higher percentage of survival.

Table 1. Days to emergence of new leaves in magic fruit cuttings applied with differentlevels of IBA 90 days after planting

Levels of IBA (ppm)	Types of Cuttings		Mean[ns]
	Softwood[1/]	Hardwood[2/]	
0	18.53[a]	31.13[ab]	24.83
200	20.86[ab]	33.46[b]	27.16
400	25.34 [abc]	28.88[ab]	27.11
600	29.74[c]	32.04[ab]	30.89
800	28.46[bc]	28.31[ab]	28.39
1000	21.28[ab]	26.78[a]	24.03
Mean[3/]	24.04[a]	30.10[b]	27.07

C.V . - 11.74%

[1/] - Means on interaction effect of softwood cuttings having the same letter superscript are not significantly different in the column at 1% level using LSD test.

[2/] - Means on interaction effect of hardwood cuttings and the level of IBA treatment having the same letter superscript are not significantly different at 1% level using LSD test.

[3/] - Main effect of types of cuttings in a row having the same letter superscript are not significantly different at 5% level LSD test.

ns - not significant

Table 2. Number of newly developed leaves in magic fruit cuttings applied withdifferent levels of IBA 90 days after planting

Levels of IBA	Type of Cuttings[ns]		Mean[ns]
	Softwood	Hardwood	
0	4.00	6.68	5.34
200	4.26	6.56	5.41
400	5.25	6.48	5.86
600	6.59	6.53	6.56
800	5.38	8.89	7.13
1000	4.42	7.66	6.04
Mean[/1]	**4.99[b]**	**7.12[a]**	**6.06**

C.V. - 29.29%

[1/] - Means followed by common letter superscripts are not significantly different at 1% levelusing T-test

ns - not significant

Table 3. Number of roots developed in magic fruit cuttings applied with different levels ofIBA 90 days after planting

Levels of IBA (ppm)	Type of Cuttings[ns]		Mean[1/]
	Softwood	Hardwood	
0	5.46	4.51	4.99[c]
200	4.99	5.11	5.05[c]
400	7.16	7.04	7.10[bc]
600	11.38	5.54	8.46[ab]
800	7.14	7.47	7.31[bc]
1000	11.14	10.36	10.75[a]
Mean[ns]	**7.88**	**6.67**	**7.28**

C.V. - 38.48%

[1/] - Means with common letter superscripts are not significantly different at 5% level usingLSD test

ns - not significant

Table 4. Percentage (%) rooting of magic fruit cuttings applied with different levels of IBA 90 days after planting

Levels of IBA (ppm)	Types of Cuttings^{ns}		Mean$^{1/}$
	Softwood	Hardwood	
0	33.33	30.00	31.67^{c}
200	50.00	36.67	43.33^{bc}
400	76.67	60.00	68.33^{ab}
600	76.67	60.00	68.33^{ab}
800	90.00	73.33	81.67^{a}
1000	36.67	46.67	41.67^{bc}
Mean^{ns}	**60.56**	**51.11**	**55.83**

C.V. - 30.73%
$^{1/}$ - Means followed by common letter superscripts are not significantly different at 1% levelusing T-test.
ns - not significant

Table 5. Percentage survival of magic fruit cuttings applied with different levels of IBA at 90days after planting

Levels of IBA (ppm)	Type of Cuttings^{ns}		Mean^{ns}
	Softwood	Hardwood	
0	76.67	86.67	81.67
200	90.00	96.67	93.33
400	93.33	96.67	95.00
600	96.67	93.33	95.00
800	100.00	90.00	95.00
1000	83.33	93.33	88.33
Mean^{ns}	**90.00**	**92.78**	**91.39**

C.V. - 11.01%
ns - not significant

CONCLUSION AND RECOMMENDATIONS

Based on the above finding, increasing rates of IBA from 200 to 800 ppm promoted higher percentage rooting on both types of cuttings, but 800 ppm of IBA gave the highest percentage of 81.67%, which was 50% higher than untreated cuttings. While softwood cuttings responded favorably to application of IBA in terms on the number of days to emergence of new leaves and number of roots developed in magic fruit cuttings. Development of new leaves was shortened to 18-25 days, while in hardwood cuttings it was delayed to 30.10 DAP.

No significant differences were obtained on the length of roots and percentagesurvival of magic fruit cuttings. Highest percentage of rooted cuttings at 30 DAP was obtained in softwood treated with 800 ppm of IBA (67.0%), while hardwood cuttings at the same treatment got 33.0% only. At 60 DAP, both softwood and hardwood from cuttings treated with 800 ppm of IBA gave 100% rooted cuttings. At 90 DAP all treatments from softwood cuttings got 100% rooting.

In view of these results, rooting of magic fruit cuttings is highly recommended using IBA with the rate of 800 ppm. If similar studies be conducted, it is recommended to use different rooting media and root-promoting hormones in stem cuttings.

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No potential conflict of interest was declared by the author.

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