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Influence of alpha-naphthalene acetic acid (ANAA) on *lubeg* (*Syzygium* spp.) marcots

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Key words: *Lubeg*; *Syzygium* spp., Callus, Root hormones, Marcot.

Abstract

The study was conducted to evaluate the effect of different Alpha - Naphthalene Acetic Acid (ANAA) concentrations to improve marcotting multiplication of *Lubeg* (*Syzygium* spp.) as planting clones in Cagayan State University at Lal-lo, Cagayan, Philippines. The study aimed to determine the influence of Alpha Naphthalenel Acetic Acid (ANAA) on the onset of callus formation, root initiation, number of roots, root length and survival rate. The marcotting media used are: Treatment 1 (no ANAA); Treatment 2 (0.5ml ANAA + 100ml distilled water at recommended rate); Treatment 3 (50% ANAA + 50% distilled water) and Treatment 4 (75% ANAA + 25% distilled water). These treatments were randomly assigned to seven experimental blocks where each available *Lubeg* mother tree was treated as a block in a Randomized Complete Block Design (RCBD). Results showed that there were significant effects on different ANAA concentrations on callus formation and length of roots. However, no significant effects on different ANA concentrations on the root initiation, number of roots and survival rate. The results showed that Lubeg (*Syzygium* spp.) exposed to root hormones were more prolific in developing root and calluses. The survived rooted cuttings manifested healthy pinkish to whitish roots. There were noticeable differences in the root or callus formation and survival of rooted cuttings in terms of different rate of ANA hormone application. It is recommended that: (a) another trial must be conducted to confirm and give more conclusive results; (b) further research and observations must be performed using different media; and (c) different types of rooting hormone. An attempt to do experiment on established rooted marcots must be conducted to determine age of maturity and height of the trees before it bears its fruit over time.

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Introduction

Lubeg (*Syzygium* spp.) is commonly grown in the province of Lal-lo, Cagayan known locally as *malubeg* and *alebadu*. *Lubeg* is good to the health of consumer because it contains anti-cancer properties and vitamin C that is essential to one's health. *Lubeg* does not need any treatment in order to bear flowers and fruit until ripening. Hence, it undergoes natural processes until it reaches maturity stage.

Lubeg is considered as one of the hidden assets of the province of Cagayan Valley. It is a fruit bearing tree and belongs to the family Myrtaceae that exists in most backyards in the locality. It is very common and well known by Cagayanos because many products can be derived from it. Matured, fresh and ripened fruits can be preserved and made as wine, vinegar and used as ingredients in *sinigang* fish or even meat due to its sour taste. This particular tree is believed to be found only in Cagayan Valley region. This is considered as one town one product (OTOP) of Lal-lo, Cagayan. In fact in 2016, a survey conducted by de Guzman, in her unpublished research found that there are more than 3,996 grown and bearing fruits all over the municipality. The highly productive trees with more than 12-14 meters in height are producing fruits twice a year ranging from 43.50–52.85kg. Tree $^{-1}$ (Molina, 2016).

According to Gonzales, AT *et al* (2016) morphologically, *Lubeg* which is endemic tree of Cagayan Valley region, is a medium – height tree ranging from 6 to 12.75 meters and a canopy spread of 9.72 meters and a trunk diameter of 48.30cm. It bears fruit twice a year with a very high yield reaching to more than 96kg per harvest/year. The fruit is spheroid to globose berry with the size ranging from 25.9 to 37.6mm and weight of 13.67g. *Lubeg* is a fruit of limited distribution in the Philippines and believe to be endemic of Cagayan Valley Region. It is a good example of a tree that is noticed only during its fruiting season and is practically neglected the rest of the year. A favorite fresh fruit among Cagayanos, it may be developed as potential industry, a potential money earner for the country because it can be processed commercially into different food products.

A total of 3,936 fruit bearing *Lubeg* trees are found planted and grown vigorously, naturally and endemically in the region. These *Lubeg* trees can produce 384,783.36kg fresh *Lubeg* fruits in a year that can be used as raw materials for wine, vinegar and other by-products, thus a good source of income of Cagayanos. Normally, the plant is sexually propagated, with the fallen fruits, seeds germinate naturally.

Marcotting may be employed as a micro-propagation technique to enhance the rapid multiplication of tall, high-yielding and purposely transform it into short-statured, early maturing *Lubeg* tree in the future.

Basically, fruit crops can be propagated sexually by seeds or asexually by stem, buds, roots, suckers or other plant parts. Seedling materials are highly variable in terms of fruit quality and yield. Long juvenility is another common characteristic of seedling trees. The traditional methods of vegetative propagation for fruits are budding and marcotting. Budding technique is commonly used for *durian*, mango and *rambutan* propagation whilst marcotting is mainly adopted for many fruit trees. Marcotting is one of the oldest forms of plant propagation. It is an asexual or vegetable method of plant propagation and can be easily performed with less skill. In this method, the induction of root development is usually done by wounding the part of the plant to be rooted. It is a method of propagating a fruit tree from an existing one, which will bear fruits sooner and the fruits will taste same as the mother plant.

Marcotting is a vegetative method of plant propagation that involves the development of aerial roots while still attached to the parent plant. The formation of roots on the layers, also referred to as marcots, require continuous moisture, adequate aeration and moderate temperatures. Although other methods of propagation exist, marcotting is preferred as it ensured more rooting success, including clones which will not root easily. In addition, marcotting is simple to perform and it allows for larger plants which are readily mature to be produced in faster time. The principal advantage of marcotting is the successful roots development from its stem.

Many clones whose cutting will not root easily can be propagated by marcotting, enabling the plants to be established on its own roots, get the exact replica of parent plants, fast fruiting time, low mortality rate, source of income and cheap. There is also a disadvantage of marcotting like diseases or defects from parent plants could be transferred to new plants. But this can be avoided by selecting a healthy, matured plant as specimen for propagation. To enhance asexual propagations, rooting hormones are usually mixed to media. A rooting hormone is a substance that encourages the plant to produce root cells. But used incorrectly they can actually inhibit root growth; however, they are often used to help the establishment of woody materials such as in heel cuttings. Alpha Naphthalene Acetic Acid (ANAA) is a growth hormone used to enhance or regulate growth and development of a plant. It acts as a catalyst for the new roots and protects the newly marcotted plants from fungus and disease that may have been introduced during the process. Applying root promoting substances, such as ANAA, during marcotting is beneficial. Hence, this study was conducted to determine the performance of marcotted *Lubeg* as influenced by different concentrations of Alpha Naphthalene Acetic Acid (ANAA).

Normally, the *Lubeg* plant is sexually propagated, with the fallen fruits, seeds germinate naturally. At present, no attempted has been made to propagate the tree asexually. Thus this context, the researcher attempts to propagate the endemic tree through marcotting technique.

Materials and methods

The experiment was carried out in fully grown 10-20 years old Lubeg trees found at the CSU Lal-lo Campus, Lal-lo, Cagayan, Philippines.

Plant and Shoot Selection

A matured branch or shoot with plenty of leaves was chosen from a healthy *Lubeg* stem. The size of stem which is about an approximate pencil size diameter was tagged as sample stem.

The Randomized Complete Block Design (RCBD) was used in the study with four (4) treatments replicated seven (7) times.

The following ANAA concentrations were as follows: T_1 – No ANAA (Control); T_2 – RR (0.5ml ANAA + 100 ml distilled water); T_3– ½ concentrations of commercial ANAA (50% ANAA + 50% distilled water), and T_4– ¾ concentrations of commercial ANAA (75% ANAA + 25% distilled water).

The coconut coir dust as marcotting media were soaked in clean water for 24 hours. Fifty-four grams of soaked coconut coir dust were used per sample (Fig. 1.b). The tagged stem were marcotted with the following standard procedure: a ring of bark around the base of the stem was scraped but not too deep into the wood (10-15cm away from the base or fork of the shoot/branch and about 3 cm above the first one) to remove the soft material and was applied with the different commercial concentrations of ANAA (treatments) with the use of ordinary paint brush. The cut surface was wrapped with soaked coconut coir dust. Marcots were properly tied to avoid spillage of the rooting medium and water.

The marcoting procedure was done after the methods of Antoniraj and Libunao *et al*. 2013, as follows:

For the care maintenance, the occurrence of pathogenic fungi was prevented with the application of 10ml of Dithane solution at the rate of one (1) tbsp./li.$^{-1}$ through injection into the marcotted *Lubeg*.

The data gathered were as follows: (1) Onset of the callus formation, this was taken by observing the number of days the callus was formed after marcotting; (2) Root initiation, this was taken by observing the number of days the first root growth was formed after marcotting; (3) Number of Roots, this was taken by counting the number of roots at the end of research; (4) Length of Longest Roots , this was taken by measuring the length of the longest roots of marcotted *Lubeg* at the end of the research using foot rule in (cm). (5) Total Number of successfully survived marcots, this was taken by computing the total number of marcotted *Lubeg* regardless of treatment and (6) Survival Rate,

this was taken by computing the percentage survival of marcotted *Lubeg*. Using the formula:

$$Survival\ Rate\ (\%) = \frac{number\ of\ marcotted\ lubeg\ survived}{number\ of\ marcotted\ lubeg}\ X\ 100$$

The data was analyzed using the Analysis of Variance (ANOVA) of the Randomized Complete Block Design.

Fig. 1. Marcotted *Lubeg* showing (a) callus formation; (b) Root initiation and formation; (c-d) root growth and color.

Results and discussion

Onset of Callus Formation

Table 1 and Fig. 4 show the effect of Different Concentrations of ANAA on the onset of callus formation. Marcotted *Lubeg* treated with ½ concentrations of commercial ANAA (50% ANAA + 50% distilled water) and with ¾ concentrations of commercial ANAA (75% ANAA + 25% distilled water) formed earliest callus (30 days) as compared to *Lubeg* without ANAA and at recommended rate of .5ml/100ml that produced callus at 35 and 39 days after treatment, respectively.

Root Initiation

The effect of different concentrations of ANAA on root initiation was numerically significant. Marcotted *Lubeg* treated with ½ concentrations of commercial ANAA (50% ANAA + 50% distilled water) was the earliest to initiate roots (61 and 61 days) followed by ¾ concentrations of commercial ANAA (66 days) (Table 1). Marcotted *Lubeg* treated with ½ strength significantly formed earliest callus and initiated earliest roots as compared to those without ANAA treatment. This is in consonance with the findings of marcotted pumelo conducted by Libunao in 2013, that marcotted pummelo treated with full, ¾ and ½ strength significantly formed earliest callus and initiated earliest roots as compared to those without ANAA treatment.

Also with Gatmen and Libunao (2006) about marcotting that show the use of 200 ppm ANAA solution had more roots observed in pummelo marcots with the coconut coir dust as rooting media. This fast root appearance and growth is thought to be due to the medium's good water holding capacity and the use of liquid rooting hormone which provided ideal conditions for good root growth. Field conditions during the time of the experiment were also favorable with high relative humidity (< 80%) and temperatures 30 – 32°C.

Number of roots

Result revealed that marcotted stem significantly produced *increasing number of* roots as the concentrations of ANAA increases. No significant differences are observed among the treated stems. Marcotted *Lubeg* treated with ½ and ¾ strength, respectively significantly gave higher number of roots than roots that received .5ml ANAA and no ANAA at all. Results show that the number of roots produced was significantly increased by application of ANAA.

This might be due to the higher concentrations of ANAA received by the plant that resulted to rapid cell division in the cambium and the tissues are active resulted to earliest callus formation and root initiation. Auxin-promoted cell division and cell expansion closely sequenced within the same tissue (root initiation, plant growth). In a living plant it appears that auxins and other plant hormones nearly always interact to determine patterns of plant development. (Interchem Technologies, 2003-2006).

The result of this experiment is in consonance with the claims of many authors about marcotting study. In the study of Libunao in 2013, for example, as to the number of roots, marcotted pummelo treated with full concentrations of commercial ANAA had significantly produced more roots with 13.45 but also comparable to marcots treated with 3/4, ½ and ¼ concentrations of commercial ANAA, with 12.63, 12.67 and 9.67 roots, respectively. According to their experiment marcotted *pummelo* not treated with ANAA had the least number of roots produced.

This implies that marcotted *pummelo* treated with ANAA had produced more roots than untreated samples. It is a well-established fact according to Edmund (1978) that IAA, IBA, ANAA are chemicals that speed up the healing of the wounds and production of roots. The chemicals also induced the development of more roots.

Length of Longest Roots

The table shows the effects of different concentrations of ANAA on root length of marcotted Lubeg. The root length of the lubeg marcots did not vary significantly. Treated marcots with ½ to ¾ concentration of commercial ANAA (75% ANAA + 25% distilled water) gave comparable length of roots to marcots treated at recommended rate and marcots without ANAA. Thus, as to length of roots, all of the marcotted *Lubeg* stem seemed have comparable length of longest roots except for marcotts treated with ANAA at recommended rate. The results show that the length of longest roots was not significantly increased by application of ANAA. This implies further that the rate of ANAA used did not cause a significant increase on root elongation. Taiz & Zeiger (1991) stated that the control of root elongation growth is not well understood. According to them, it was hypothesized that the auxin in the roots is much lower. It also implies that the temperature during the conduct of the study was favorable resulted to roots produced which conforms to the statement of Bryant (2003) as reported by Obille (2007) that the warmer the temperature, the rate of physiological processes increased.

Survival Rate

Results show no significant differences observed on the percentage success of marcots and survival rate as an effect of different commercial strength of ANAA application to marcots. The result revealed that the different levels of concentrations of ANAA did not influence the percentage success of marcots and survival of marcots. This implies that survived *Lubeg* treated with different concentrations of commercial ANAA is comparable to marcotted *Lubeg* with no ANAA.

The survival rate of marcots showed comparable higher percentage success with a 97.14% in marcots treated with ANAA at recommended rate followed with *Lubeg* treated with 50% ANAA. But it was observed that the lowest survival rate was registered in *Lubeg* treated with higher concentrations of ANAA. This implies that the greater the roots produced of marcots the greater the assurance of survival due to the fact that the roots system absorbed more foods, nutrients and uptake of water from the soil to the different parts of the plants.

This is in consonance with the findings of Kowzlowski and Kramer (1960) that the more the roots produced the more water and minerals that will sustain the survival of the sprouts. This attributes to the function of the root system on the general absorption of food and uptake of water from the soil to the different parts of the plants.

The result of study corroborates with the work of Castillo (2001) on marcotting on durian showed that the rooting hormone affected the percentage of rooted branchlet. The 300 ppm concentration of IBA performed better in terms of rooting ability compared to the lesser ones. Hormone also influenced the number of roots and length of adventitious root. A combination of cutting age and/or the extent of lignification in the cuttings are also important factors in successful root formation from cuttings (Cameron *et al.*, 2003).

Table 1. Onset of - the callus formation, root initiation, average number of days to root initiation, length-- of-longest—roots and survival rate of- marcotted-*lubeg*- as affected by different concentrations of ANAA.

Treatment	Onset of Callus Formation (Days)	Root Initiation (Days)	Average number of days to root initiation	Number of Roots	Means (cm)	Survival rate (%)
T_1- No ANAA (Control)	39 a	81 a	62.29 a	1.24 b	3.10	94.29
T_2- Recommended Rate (0.5ml ANAA +100ml distilled water)	35 b	72 b	40.86 c	2.01 b	3.19	97.14
T_3- ½ concentration of commercial ANAA (50% ANAA + 50% distilled water)	30 c	61 c	53.86 b	5.29 a	3.12	94.29
T_4- ¾ concentration of commercial ANAA (75% ANAA + 25% distilled water)	30 c	66 bc	46.86 c	5.78 a	3.71	97.43

*All means followed by the same letter are not significantly different at 5% level, LSD.

In addition, Lopez (1975) reported that the use of rooting hormones in breadfruit marcotting and stem cuttings under controlled environment and found out that 95% of the marcotts and cuttings producing sufficient root, shoot growth and stem elongation after ten weeks. This early growth, in his findings, coincided with the highest levels of stored carbohydrates in the mother plant and it inhibited the development of lateral buds allowing the new plant maintain apical dominance. Hamilton *et al.* (1982) also demonstrated that the use of rooting hormones in marcotting of breadfruit increased rooting. He explained that this was due to the action of the hormone, Indole Acetic Acid (IA) in root growth and development. In addition, Cerveny and Gibson (2005) studied the effectiveness of powdered rooting hormones and liquid hormones on stems cuttings. In that work, they found powdered forms of rooting hormones generally less effective than liquid formulation applied at the same concentration. They further added, marcotting media with rooting hormone facilitate rooting, where cultural practices or environmental conditions are not ideal and also in the propagation of moderate and difficult-to-root species. In a more recent work, the FAO-UN (2012) reported a study on the effects of marcotting media and branch

size on rooting and that was carried out in China, Thailand, India, and Bangladesh showed that the medium consisting of 100 per cent peat moss and medium mixed with rooting hormone improved rooting of marcotts and resulted in more than 90% of the success rate.

In addition, the climatological data in CSU Lal-lo, Cagayan, Philippines during the conduct of the study is considered favourable for marcotting for *Lubeg* because according to Dirr and Hueser (2000) warm temperature promotes callus formation; cool temperature inhibits or reduces it, while extremely high temperatures are detrimental. The highest temperature of 30°C was recorded in March and lowest temperature ranged from 22°C. The total rainfall was 8.36cm the highest was recorded in January (11.8cm).

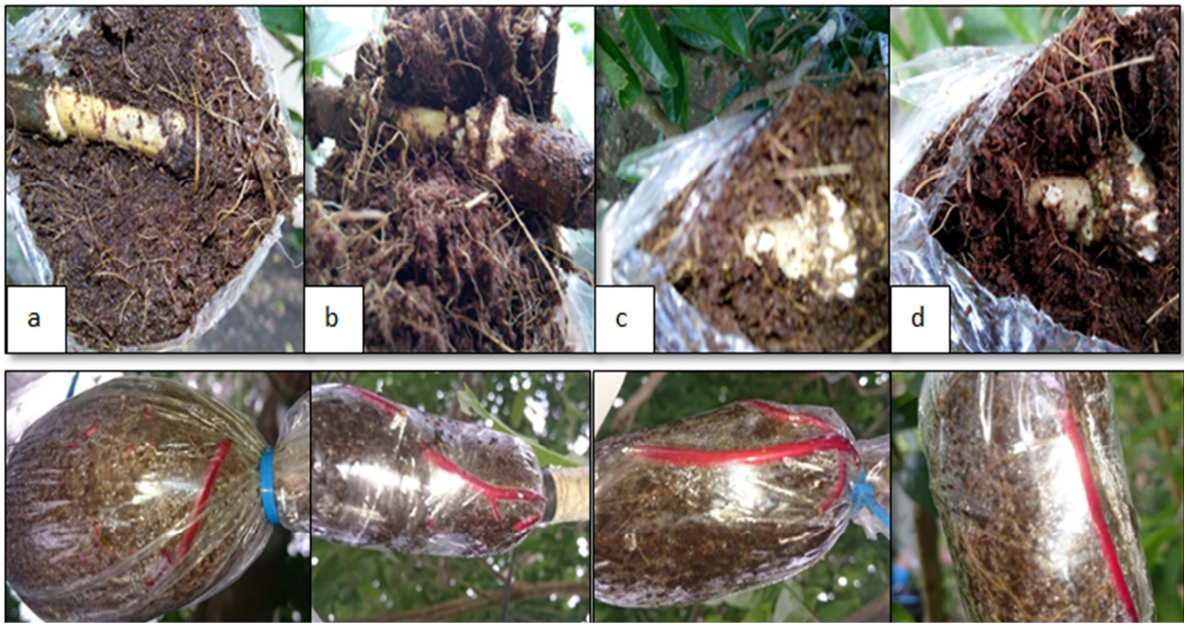

Fig. 3. Marcotted *Lubeg* treated with Different Concentrations of ANAA showing callus formation: (a- T1; b- T2; c –T3 and d T4).

Summary, conclusions and recommendation

Lubeg plant, an endemic of Lal-lo, Cagayan is erect and heavily producing perishable fruits. It is normally sexually propagated, with the fallen fruits, seeds germinate naturally. At present, no attempt has been made to propagate the tree asexually. In this context, the researcher attempted to propagate the endemic tree through marcotting technique to evaluate the effects of different concentration Alpha - Naphthatene Acetic Acid (ANAA) on *Lubeg* (*Zysygium* spp.) to improve marcotting multiplication of planting materials in Cagayan State University at Lal-lo, Lal-lo Cagayan. Marcotting may be employed as a micro-propagation technique to enhance the rapid multiplication of tall, high-yielding and purposely transform it into short-statured, early maturing *Lubeg* tree in the future. In this study, marcotting was performed on *Lubeg* utilizing coconut coir dust planting media, with different rates of rooting hormone (active ingredient: Alpha Naphthalene Acetic Acid or ANAA.

The preliminary trial aimed to determine its effect on the onset of callus formation, first root initiation, number of roots, root length and survival rate, and total number of successfully survived marcots. The marcotting media used were: Treatment 1 (no ANAA); Treatment 2 (0.5 ml ANAA + 100 ml distilled water, recommended rate); T_3 (50% ANAA + 50% distilled water) and T_4 (75% ANAA + 25% distilled water). These treatments were randomly assigned to seven experimental blocks where each available *Lubeg* tree was treated as a block in a Randomized Complete Block Design (RCBD).

Results showed that there were significant effects of different ANAA concentrations on the callus formation, root initiation; number of roots and survival rate. However, marcots treated with different levels of ANAA concentrations did not differ significantly in terms of length of roots. There were noticeable differences in the root or callus formation and survival of rooted cuttings in terms of different rate of ANAA hormone application. This promising results showed that *Lubeg* (*Zysygium* spp.) exposed to root hormones was more prolific in developing root and calluses, thus can be mass produced asexually through marcotting technique. The survived rooted cuttings manifested healthy pinkish to whitish roots. It is recommended that: (a) another trial must be conducted using different media; (b) different types of rooting hormone must be evaluated. An attempt to do experiment on established rooted marcots must be conducted also to determine age at maturity and height of the trees over time.

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