



## RESEARCH PAPER

## OPEN ACCESS

## Root initiation of Lipote (*Syzygium polycephaloides* (C.B.Rob.) Merr.) cuttings using growth hormones

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**Key words:** Lipote, IBA, Hormex, *Syzygium polycephaloides*, Growth hormones

<http://dx.doi.org/10.12692/ijb/22.4.140-147>

Article published on April 22, 2023

### Abstract

Lipote (*Syzygium polycephaloides*) is a Philippines indigenous tree. Despite its nutritional benefits, there were only limited researches about its propagation. Thus, this study investigated the effect of growth hormones (IBA and Hormex) on lipote cuttings. The study consisted of ten treatments that laid-out in two factorial Complete Randomized Design (CRD), wherein factor A were the two different growth hormones and factor B were the various concentrations. Each treatment was replicated by four and 8 cuttings per treatment. The study was terminated after 45 days. Results revealed that the interaction between growth hormones and level of concentrations had a substantial effect on percent shooting and percent callusing of lipote cuttings, but no significant differences were found on percent survival, percent rooting, number of adventitious roots, mean shoot length and number of shoots.

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## Introduction

Native trees play a very important role for sustaining the abundance of the biodiversity of our ecosystem. They are fundamental to our natural ecosystems as they retain their natural capacity to form devastation caused by raging weather and from pests and diseases. Native trees also adapt naturally to their local surrounding, thus more resilient than introduced species (DOST, 2015). In recent decades, there has been a sharp decline in the population of native trees caused by destructive and extractive human activities. The main reasons of the declining number of native trees are the destructive deforestation, plating of invasive alien species, and monocrop plantations that propagate only commercially popular varieties (Cabansag, 2016).

Not all trees are easily propagated by seeds and the idea of vegetative propagation was considered in order to produce quality seedlings. Through clonal propagation, a significant amount of high-quality planting materials can be produced in a short period of time (Wetzstein *et al.*, 2018). Indole butyric acid was discovered to significantly stimulate root formation of cuttings. Natural auxins respond to the injured area and cause rooting to begin. Exogenous application of IBA on cuttings can accelerate the rooting and increases the percent rooting and the number of roots per plant. IBA also promotes cell division, cell elongation, and metabolic activity at the site of the incision (Rymbai *et al.*, 2010). Apart from using IBA for clonal propagation, Hormex rooting hormone concentrate and vitamin B1 is also known as rooting product that can root new plants from cuttings easily. The auxin known as IBA (indole-3-butyric acid), ANA (alpha-naphthalene acetic acid) and Thiamine hydrochloride contributes to the formation and growth of healthy roots and these are the active ingredients of Hormex rooting hormone concentrate and vitamin B1 (Hormex, 2021).

Lipote (*Syzygium polycephaloides*) is a Philippines indigenous tree. The occurrence of lipote in its natural habitat is currently scarce and has been included in Madulid (2000) list of rare and vanishing fruit trees and shrubs in the Philippines because it is

barely seen in its natural habitat. According to Coronel (2011), ripe fruits of *Syzygium polycephaloides* are consumed fresh by local people. The mature fruit, which is high in vitamin C, is used to make jams, jellies, and juice. The juice of lipote is fermented into wine. The fruit's flesh is dehydrated and utilized for pills or capsules. Folkloric and medical uses include the treatment of diabetes, hypertension, and excessive cholesterol.

With proper utilization of the rooting hormones (IBA and Hormex), it is expected that it will give better effect to cuttings of lipote, which is a potential tree crop for agroforestry and reforestation. Therefore, the ultimate objective of this study is to assess the rooting ability of varying concentration of IBA and Hormex solutions in lipote cuttings.

## Materials and Methods

### *Collection and Preparation of the cuttings*

The cuttings of lipote were collected from a 2-year-old Lipote tree located at Magapuy, Bayombong, Nueva Vizcaya. Healthy cuttings containing three buds were cut from stock plants of Lipote in slanting using a pruning shear. These Lipote cuttings were collected early in the morning to minimize loss of moisture through transpiration. The cuttings were bundled together and immediately submerged in separate basins of tap water to washed off the foreign matter and dust, as well as insects and other microorganisms. The leaves of lipote were trimmed to half of their original size to prevent excessive transpiration, rot, and for ease of dibbling them into the rooting medium. After that, the cuttings were soaked into a 200-ppm fungicide (Dithane M-45) solution for one hour to eliminate fungal contamination.

Prior to growth hormone application, the cuttings were bundled into 10 pieces then the base portion was scraped using sharp knife to ensure that the cuttings are of the same length and the base parts will be of the same end when dipping them into the prepared rooting hormone. It could facilitate even surface contact and the distribution of rooting hormone to the basal end of the cuttings.

### Preparation and Application of Rooting Hormones

The hormex rooting hormone is a solution and the IBA rooting hormone was prepared from powder form. A digital weighing scale was used in weighing one (1) gram of the IBA then dissolved in a 5 ml of sodium hydroxide. The two rooting hormones were volumeter to 1000 ml by adding 999 ml of distilled water in 1 ml of IBA solution using a volumetric flask. The resulting solution produced a stock solution of 1000ppm concentration (Quinan, 2019). The varying concentrations of 250ppm, 500ppm and 750ppm were prepared through serial dilution of the stock solution. On the other hand, pure distilled water was used as a control with varying measurements of 250ppm, 500ppm, 750ppm and 1000ppm. All the preparation of rooting hormones was done at the Tissue Culture Laboratory of Nueva Vizcaya State University. After thorough preparation of the Lipote cuttings, 1.0 cm of the basal part of each bundled cutting was dipped into the IBA and Hormex root solutions for 1 hour, except for the control, which was dipped into distilled water only.

### Data Collection

The data collection for rooting initiation was 45 days when the majority of the cuttings produced roots. All the cuttings were taken out of the propagation chamber and the harvesting of individual plants was done carefully by gently pulling out the cuttings from the planting beds and removing the soils that clings to the individual plant. Soils that adhere to the plant roots were washed-off by gently flushing them with running water to manage the measurement of root length and determine the number of roots in each plant as well as the formed callus.

### Location and Duration of the Study

The study was conducted at Nueva Vizcaya State University- Main Campus, College of Forestry, Environment and Resources Management Nursery, Bayombong, Nueva Vizcaya (Fig. 1). The duration of the study were four months. The rooting experiment started last January 4, 2022 up until February 18, 2022.

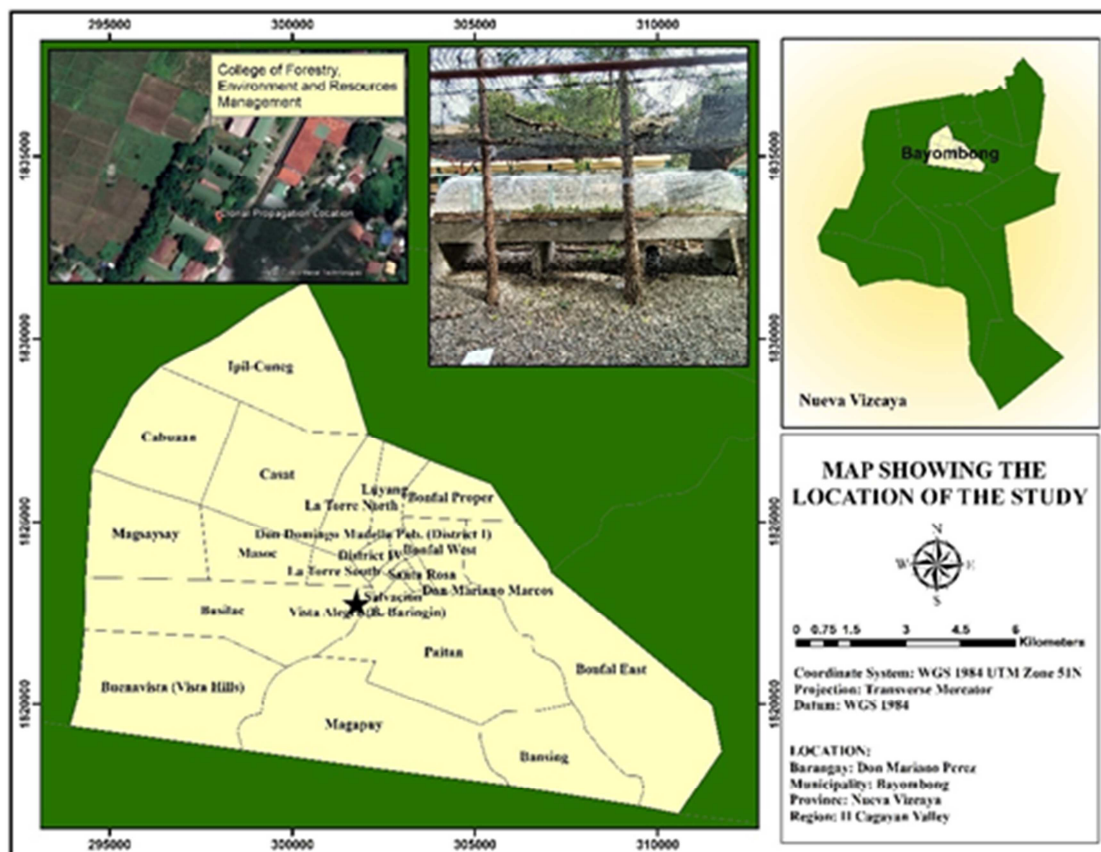


Fig. 1. Location of the study.

### *Experimental Design*

The study was a 2x5 factorial experiment in Completely Randomized Design (CRD), consisted of 10 treatments, replicated 4 times, and 8 seedlings per replication, for a total of 320 experimental units. Factor A was the rooting hormones (IBA and Hormex) and Factor B was the varying concentrations (0ppm, 250ppm, 500ppm, 750ppm and 1000ppm).

### **Results**

#### *Effect of Rooting Hormones on Lipote Cuttings*

The survival rate of lipote cuttings was not found significantly impacted by the rooting hormones (Table 1). In accordance to the result, the cuttings applied with IBA (7.81%) had higher percent survival than Hormex (2.5%). IBA may had higher result than hormex, but it is still considered as low survival rate.

The rooting hormones did not also significantly affect the rooting parameters of lipote cuttings such as percent rooting, number of adventitious roots and length of adventitious roots (Table 1). In percent rooting, the cuttings applied with IBA treatment (8.58%) had higher rooting percentage than Hormex (3.75%). The rooting percentage of the cuttings applied with rooting hormones were significantly low. This implies that the ability of lipote to produce root is not dependent on rooting hormones. Same with number of adventitious of lipote cuttings that IBA (0.33mm) had higher mean number of a roots than cuttings treated with Hormex (0.05mm). Longest length of roots was found in cuttings treated by IBA with mean root length of 6.53mm and cuttings treated with Hormex obtained mean length of 2.23mm.

The effect of rooting hormones on shoot percentage of lipote cuttings revealed significant differences, but not with number of shoots and length of shoots (Table 1). Analysis of variance showed that rooting hormones was significantly affect the shoot percentage of lipote cuttings. In accordance to the results, cuttings treated with Hormex (38.13%) attained higher shoot percentage as compared to cuttings that applied with IBA (21.25%).

No significant differences were found on number of shoots and length of shoots of lipote cuttings, but

results still revealed that Hormex had higher results than IBA. In number of shoots, cuttings applied with Hormex had a minimum number of 0.56 and IBA had 0.33. For the mean length of shoots, cuttings applied with Hormex obtained 3.49mm and IBA had 1.91mm (Table 1). The application of rooting hormones had significant effect on callus percentage of lipote cuttings. In accordance to the result, cuttings applied with Hormex (46.25%) had higher callus percentage than cuttings that received IBA (24.38%) (Table 1).

#### *Effect of level of concentrations on Lipote Cuttings*

Analysis of variance revealed that level of concentrations did not significantly affect the percent survival of lipote cuttings (Table 1). Highest result of percent survival rate was found in concentration of 500ppm (10.94%), followed by 250ppm (4.69%) while 750ppm of concentration attained zero percentage of survival. Even 500ppm concentration had higher results, control (3.13%) still competed well with other concentration of rooting hormones.

The level of concentrations had significant effect on root length of lipote cuttings but not with percent rooting and number of adventitious roots ((Table 1)). In root length, longest root was obtained in cuttings that received 500ppm concentrations (13.88mm) and followed by cuttings treated with 250ppm concentration (5.31mm). In percent rooting, highest rooting was found in 500ppm concentration (14.06%), followed by 250ppm (6.25%), while 750ppm obtained zero rooting percentage because all the cuttings were all dead. In mean number of adventitious roots, highest number was found in 500ppm concentration (0.66mm) and followed by 250ppm concentration (0.23mm).

The shoot production of lipote cuttings was not found affected by level of concentrations (Table 1). The highest percent shooting was found in control (43.75%), followed by 500ppm and 1000ppm concentrations with 32.18%, and the lowest shoot percentage was obtained in 250ppm concentrations (17.19%). In mean number of shoots, highest was found in 500ppm concentration (0.60) followed by the 1000ppm concentration and control with both

mean number of 0.5. In shoot length, longest (3.53mm) was obtained in 500ppm concentration, followed by the control (3.42mm) and 1000ppm concentration (3.25ppm).

The level of concentrations of respective rooting hormones had significant effect on callus percentage of lipote cuttings (Table 1). Highest (48.44%) callus percentage was found in control.

#### *Interaction Effect of Rooting Hormones and Level of Concentration on Lipote Cuttings*

Analysis of variance showed that the interaction between rooting hormones and concentration had no significant effect on percent survival of lipote cuttings (Table 1). Highest survival rate was found in 500ppm of IBA (18.75%) while zero survival rate were found in 750ppm and 1000ppm of Hormex treatments and in 750ppm of IBA.

Percent survival (%)	Percent callusing (%)	Shoot length (mm)	Mean Number of shoots	Percent shooting (%)	Root length (mm)	Number of adventitious roots (mm)	Percent rooting (%)	Treatments
3.13%	43.75 a	2.97	0.4	37.50% ab	1.88	0.03	3.13%	H1C1- Control
6.25%	12.50 b	1	0.13	12.50% b	2.56	0.06	6.25%	H1C2- 250ppm Hormex
3.13%	62.50 a	4.47	0.72	31.25 ab	8.06	0.16	9.38%	H1C3- 500ppm Hormex
0%	46.875 a	3.75	0.84	43.75% a	0	0	0%	H1C4- 750ppm Hormex
0%	65.63 a	5.13	0.78	56.25% a	0	0	0%	H1C5- 1000ppm Hormex
3.13%	53.13 a	3.88	0.59	50% a	2.81	0.03	3.13%	H2C1- Control
3.13%	28.13 abc	1.72	0.38	21.88% ab	7.66	0.41	6.25%	H2C2- 250ppm IBA
18.75%	31.25 ab	2.59	0.47	25% ab	19.69	1.16	18.75%	H2C3- 500ppm IBA
0%	0 c	0	0	0% b	0	0	0%	H2C4-750ppm IBA
6.25%	9.375 bc	1.36	0.22	9.38% b	2.5	0.06	6.25%	H2C5- 1000ppm IBA
0.22	0.0053	0.18	0.06	0.03	0.78	0.32	0.79	p-value at 5%
Not Significant	Significant	Not Significant	Not Significant	Significant	Not Significant	Not Significant	Not Significant	Significant level

The interaction effect of rooting hormones and concentrations was not significant on all root parameters of lipote cuttings (Table 1). In percent rooting of lipote cutting, highest rate was 18.75% and was observed in cuttings treated with 500ppm of IBA, followed by 250ppm of Hormex (9.38%), while zero rooting from 750ppm and 1000ppm of Hormex. In number of adventitious roots, 500ppm IBA (1.16mm) had the highest mean number, followed by 250ppm IBA (0.41mm), respectively. In length of adventitious roots of Lipote cuttings, maximum root length was found in treatment 500ppm IBA with 19.69mm length and followed by 250ppm hormex.

The interaction between rooting hormones and concentrations gave significant effect on the production of shoots of Lipote cuttings but not with number of shoots and shoot length (Table 1). The highest shoot percentage was found in cuttings treated with 1000ppm of hormex (56.25%), followed

by 750ppm of Hormex, then by 500ppm of hormex (Fig.). Highest mean number (0.81) of shoots were found in cuttings treated with 750ppm of hormex, followed by 1000ppm hormex (0.78), then by 250ppm hormex (0.71) respectively. Longest (5.125mm) shoots were found on cuttings treated with 1000ppm of hormex, followed by cuttings treated with 500ppm of hormex (4.47mm), respectively. The interaction effect of rooting hormones and level of concentrations had significant effect on the development of callus of lipote cuttings ((Table 1)). The highest percentage of cuttings with developed callus was 65.63% and was found in cuttings treated with 1000ppm of hormex, followed by cuttings (62.5%) treated with 500ppm of hormex, respectively. The untreated cuttings registered high percentage of cuttings with developed callus.

#### **Discussion**

The lipote attained very low survival rate as affected by different concentrations of rooting hormones.

Lipote cuttings can still survive without IBA nor Hormex. According to Hartmann and Kester as cited in the study of Kassahun *et al.* (2011), easy to root species do not respond well to the application of rooting hormones. Araya (2005) also stated that the rooting hormones in difficult to root plants have an important role in the development of adventitious roots, increasing percentage of root and for the improvement of quality roots and uniformity of roots of cuttings. The result of conducted study was supported with the study of Benabise *et al.* (2021) that percent survival of *A. bunius* cuttings as affected by various concentrations of rooting hormones revealed no significant differences. As stated by Maile and Nieuwenhuis (2010), failure to the percent survival of cloned plants is always ascribed to a number of reasons, including the age-related physiology and morphology of donor plants, as well as their phenology, which includes elements such as plant carbohydrate and nutrient contents, as well as the presence of growth regulators. In addition, according to Hoogenboom (2022) the clonal propagation chamber should not be exposed nor frequently opened because environmental parameters such as adequate relative humidity, temperature, light and airflow should be carefully considered for the survival of cuttings.

The analysis of variance showed significant differences on root length of lipote cuttings as affected by different level of concentrations regardless of the two rooting hormones. According to Singh *et al.* (2003) the concentration level of rooting hormones can significantly help the acceleration of cell elongation and cell division of the cuttings. The result coincides with the finding of Hamidon *et al.* (2020) that there were no significant differences on root length of *M. cochinchinens* as applied with four level of concentrations of rooting hormones. In a parallel result, Gehlot *et al.* (2014) found that different level of concentrations of rooting hormones was significantly affect the root length of *Azadirachta indica*, and 250ppm concentration had the best result.

Analysis of variance revealed significant differences on shoot length of lipote cuttings as affected by

rooting hormones but not on level of concentration. According to Singh and Singh (2002) that the application of rooting hormones has some role in augmenting the production of shoots per cutting. Therefore, the application of rooting hormones might positively affect the shoot percentage of lipote cuttings because of nutrient absorption through the help of rooting hormones. The findings were supported by the results of the study of Rahman (2000) on rooting of litchi that the highest production of leaves was recorded in cuttings that received growth hormone (IBA) and the lowest was observed in control treatment. Moreover, Omer *et al.* (2015) revealed that rooting hormones had significant effect on shoot percentage of *Dalbergia* stem cuttings.

The interaction between rooting hormones and level of concentrations has significant effect on shoot percentage of lipote cuttings. As mentioned by Kuntagol *et al.* (2018), natural and synthetic auxin hormones have a positive effect on the overall growth of cuttings. These hormones increase the development of pre-existing root primordial and the number of roots per cutting, which can further help the shoot development. In a parallel result, Siddiqui *et al.* (2007) revealed that maximum sprouting percentage (43.67) was observed by treating cuttings with 4000ppm of IBA solution and minimum sprouting (5.0%) was recorded in control.

Analysis of variance showed significant effect on callus percentage of lipote cuttings as affected by rooting hormones, level of concentrations and their interaction effect. After a cutting is wounded, callus tissue forms at the base, primarily from the vascular tissue. In easy-to-root plant species, callus formation and root formation are independent processes that occur at the same time because of similar environmental triggers. In difficult-to-root species, adventitious roots arise from the callus mass (Luna and Haase, 2014). The untreated cuttings registered high percentage of cuttings with developed callus. In due course, this shown that the cuttings can easily produce callus without any treatment. In the study of Castaneto and Inhumang (2003), they showed that different level of rooting hormones had no significant

effect on callus development of Ipil stem cuttings. Similarly, Karam and Gebre (2004) research on rooting of *Cercis siliquastrum* cuttings revealed that survival and callus formation were not affected by concentration of commercial rooting hormone, these results were showed in terminal cuttings of *C. siliquastrum*.

### Conclusion

The interaction effect of growth hormones and level of concentrations gave significant differences on percent shooting and percent callusing. Moreover, the effect of rooting hormones gave significant influence on percent shooting and percent callusing, and the level of concentrations, regardless of rooting hormones, gave significant effect on root length and percent callusing. IBA treatment gave better results on root parameters while hormex had better performance on shoot development. The 500ppm IBA gave the best results on different root parameters and 1000ppm hormex had the best results on shoot parameters. In shoot parameters, the higher the hormex concentration, the better the results would be. However, in accordance with the results, the percent survival of lipote cuttings was significantly low, therefore, the rooting hormones used in this study was not needed for the root initiation of lipote. The study recommended the 500ppm IBA for the root initiation of lipote as it gave the best results in root parameters and higher concentration of Hormex for better shoot development of lipote.

### Acknowledgements

We are very grateful to Department of Science and Technology for providing financial support for this study.

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