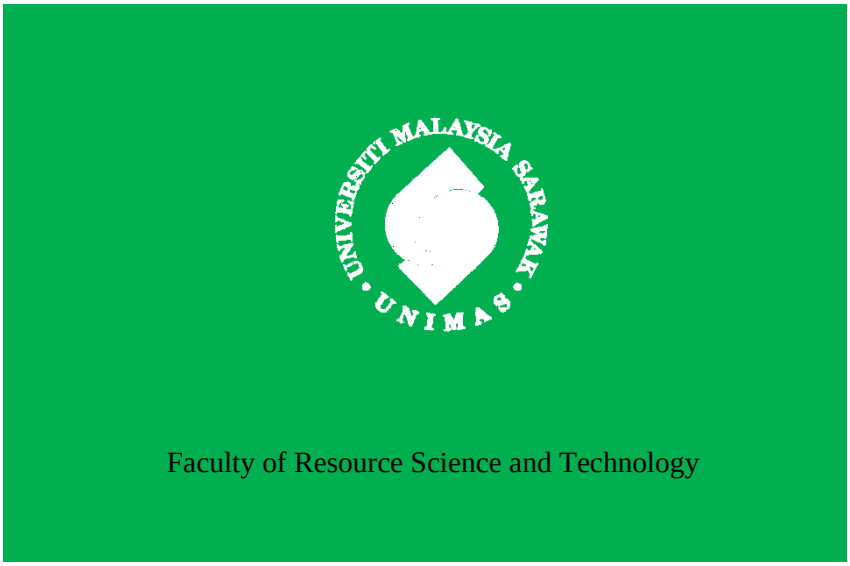

Faculty of Resource Science and Technology

Propagation of *Vanilla planifolia sp.* via Stem Cutting

Syed Zulhakimi bin Syed Zulkipli YH

Bachelor of Science with Honours
(Plant Resource Science and Management)
2022

Propagation of *Vanilla planifolia sp.* via Stem Cuttings

SYED ZULHAKIMI BIN SYED ZULKIPLI

This thesis is submitted in partial fulfilment of the requirement for the degree of the Bachelor of Science with Honors

(Plant Resource Science and Management)

Supervisor: Dr Hashimah binti Elias

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

2022

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Studies on Vegetatative Propagation of Vanilla planifolia via stem cutting

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ABSTRACT

Vanilla planifolia is an orchid species belonging to the Orchidaceae family, which ranked the second largest family of flowering plants and usually cultivated for their priceless pods or fruits, besides various beneficial compounds in the plant. The main issue for growing this plant species is the very slow rate of multiplication, their extensive time taken to fully mature, which is around two to four years. Thus, this project is focused on applying numerous possible approaches towards the *in vivo* propagation of *Vanilla planifolia* via stem cutting. The purpose of this studyis to identify the most effective hormone to enhance propagation and maximum growth of the plant. Overall, 13 cuttings were applied with ten different treatments including control, BAP (5,10,15 mg/L), GA3 (5,10,15 mg/L) and NAA+BAP (5,10,15 mg/L) The experiment wasconducted using a completely randomized design. At 30th day of planting, the number of roots produced, length of roots produced, colour of root, diameter of cuttings and survival rate were observed and recorded. Mean comparison was done for all treatments of each parameter. Nevertheless, all parameters showed insignificant differences compared to control. Further study should be done by considering other factors that could also influence the growth and development of *Vanilla planifolia* including sterilization procedures, types and concentrations of hormones, growth condition, time frame for experiment etc.

Keyword : *Orchidaceae*, hormone, BAP, GA3, NAA

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ABSTRAK

Vanilla planifolia ialah spesies orkid yang tergolong dalam keluarga Orchidaceae, yang menduduki tempat kedua terbesar bagi tumbuhan berbunga dan biasanya ditanam untuk pod atau buahnya yang tidak ternilai, selain pelbagai kompound bermanfaat dalam tumbuhan tersebut. Isu utama untuk menanam spesies tumbuhan ini ialah kadar pendaraban yang sangat perlahan, masa yang panjang diambil untuk matang sepenuhnya, iaitu sekitar dua hingga empat tahun. Oleh itu, projek ini tertumpu pada penggunaan pelbagai pendekatan yang mungkin ke arah pembiakan in vivo *Vanilla planifolia* melalui keratan batang. Tujuan kajian ini adalah untuk mengenal pasti hormon yang paling berkesan untuk meningkatkan pembiakan dan pertumbuhan maksimum tumbuhan. Secara keseluruhan, 13 keratan telah digunakan dengan sepuluh rawatan berbeza termasuk kawalan, BAP (5,10,15 mg/L), GA3 (5,10,15 mg/L) dan NAA+BAP (5,10,15 mg/L) Eksperimen dijalankan menggunakan reka bentuk rawak sepenuhnya. Pada hari ke-30penanaman, bilangan akar yang dihasilkan, panjang akar yang dihasilkan, warna akar, diameter keratan dan kadar kemandirian diperhatikan dan direkodkan. Perbandingan purata telah dilakukan untuk semua rawatan bagi setiap parameter. Namun begitu, kesemua parameter menunjukkan perbezaan yang tidak ketara berbanding kawalan. Kajian lanjut perlu dilakukan dengan mempertimbangkan faktor lain yang juga boleh mempengaruhi pertumbuhan dan perkembangan Vanilla planifolia termasuk prosedur pensterilan, jenis dan kepekatan hormon, keadaan pertumbuhan, jangka masa untuk eksperimen dan lain-lain.

Kata Kunci: *Orchidaceae*, hormon, BAP, GA3, NAA

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List of Abbreviations

%	Percentage
ANOVA	One-way analysis of variance
cm	centimeter
CRD	Completely Randomized Design
DMRT	Duncan's Multiple Range Test
et al.	et alia
BAP	6-Benzylaminopurine
GA3	Gibberellic Acid
NAA	1-Naphthaleneacetic Acid
mg/L	milligram per litre
mm	millimeter
SE	standard erro

Chapter 1: Introduction

1.1 Background

Orchids are fascinating plants that are mostly appreciated for their lovely flowers. Among all of the attractive and popular orchids, only a few species produce fruits with high economic value including *Vanilla* spp. *Vanilla* spp. are among the important orchids, cultivated for its distinct aroma and flavoring pods that widely applied especially in the food industry. *Vanilla* species is a tropical vine orchid native to Central America, southeastern Mexico, the West Indies and northern South America. (Bruman, H., 1948). Among all the vanilla orchid species found worldwide, only three species are cultivated commercially such as *V. planifolia* Andr (synonym: *V. fragrans*), *V. pompana* Scheide, and *V. tahitensis* J.W.Moore. (Castro-Bobadilla. G et al, 2007).

Genus Vanilla consists of 110 *Vanilla* species which belong to the Orchidaceae family. (Divakaran, M. et al, 2006). The most popular species is *Vanilla planifolia* (Figure 1). Besides *V. planifolia*, there are two other commercial species namely *Vanilla tahitientis* and *Vanilla pompona* which are critical species cultivated for natural vanilla flavour production. (Havkin-Frenkel, D & Dorn, R., 1997). These species have their own unique features that could be used to distinguish each other. Despite the various options of species for the production of vanilla flavour, *Vanilla planifolia* is the most preferred compared to the other two species for the quality and unique flavour. (Ehlers, D. & Pfister, M., 1997).

The problem in sexual propagation of the certain orchid species by seeds is their inability to germinate properly under normal conditions (Alomia, Y.A. et al., 2016). Therefore, another alternative methods for propagation of vanilla plants are using asexual methods such as *in vivo* via stem cutting or *in vitro* via tissue culture. However, propagating vanilla plants via stem cutting contributes to a very slow rate of multiplication and is considered not economical(Geetha, S. & Shetty SA, 2000). Thus, many studies about vanilla species are usually conducted via *in vitro* multiplication using axillary buds and shoot tips due to their advantage of reducing disturbance to the mother plant and yield reduction (Kalimuthu K. et al, 2006).

Figure 1: *Vanilla planifolia* is the most popular and cultivated species due to its high economic value.

1.2 Problem Statement

Until today, any research study regarding vanilla plants is conducted via *in vitro* propagation and very few are focusing on *in vivo* propagation, thus contributing to lack of information about effective propagation of vanilla plants via stem cuttings. Therefore, a series of studies regarding *in vivo* propagation is needed to gain new insight about the current multiplication problems and subsequently provide crucial solutions or ideas on vegetative propagation of *V. planifolia* by stem cutting.

1.3 Objective

The objective of this study is to investigate the effect of hormone application (types and concentration) on shoot and root formation of *Vanilla planifolia*. Furthermore, this study is also conducted to determine which application of different types and concentrations of the plant hormones promotes the best and effective shoot and root formation.

CHAPTER TWO: LITERATURE REVIEW

2.1 Vanilla planifolia sp.

2.1.1 Origin and Derivation

Vanilla is a tropical vine orchid native to Central America, southeastern Mexico, the West Indies and northern South America. (Bruman, 1948). The word "Vanilla" is derived from the Spanish word *vainilla* which translates the little pod, basically describing the shape of the pod itself (Ackerman & James, 2002). Vanilla has been used worldwide as a term to describe the flavor which has been commercially used in the food industry (Yang, Z. et al, 2014).

2.1.2 Taxonomic classification of *Vanilla planifolia*

Kingdom Plantae (plantes, Planta, Vegetal, plants)
Subkingdom Viridiplantae (green plants)
Infrakingdom Streptophyta (land plants)
Superdivision Embryophyta
Division Tracheophyta (vascular plants, tracheophytes)
Subdivision Spermatophytina (spermatophytes, seed plants, phanérogames)
Class Magnoliopsida
Superorder Lilianae (monocots, monocotyledons, monocotylédones)
Order Asparagales
Family Orchidaceae (orchids)
Genus Vanilla Mill. (vanilla)
Species *Vanilla planifolia* Jacks. ex Andrews (vanilla, Vainilla tlilxóchil)

(Retrieved from: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=43719#null)

2.1.3 Nutritional and Commercial Value

V. planifolia is the most popular and cultivated globally. The fruits or pods (Figure 2) produce sweet aroma and flavour which has been extensively applied in many industries including food and beverages, chocolate and dairy products, confectionery, pharmaceuticals, perfumery, cosmetics etc (Baqueiro-Pena, I & Guerrero-Beltran, J.A., 2017). It is considered as the most popular flavour in the world and second most expensive spice next to saffron (Abebe, Z. et al, 2009).

Figure 2: Fruits or pods of *V. planifolia* produce sweet and distinct aroma and flavour.

2.2 Propagation technique

2.2.1 *In vitro* propagation

As other plant species, vanilla can also be multiplied and propagated. They can be propagated either by seeds, tissue culture or stem cutting. Effective and efficient propagation of *Vanilla planifolia* is essential as it contributes to mass production of vanilla pods for commercial purposes. Ab Rahman Zuraida et. al. (2013) suggests that the most simple and efficient method for mass propagation of *Vanilla planifolia* is by *in vitro* propagation where the study described a direct shoot regeneration-based micropropagation procedure for *Vanilla planifolia*. It was concluded that the stem nodal segments showed higher potential to regenerate compared to shootapices.

2.2.2 *In vivo* propagation

Stem cutting is one of the propagation methods that is applicable to the vanilla species. Adugna, M. et al. (2015) have conducted a study to investigate the effect of rooting media and number of nodes on nursery performance of *Vanilla planifolia.* The medium used are fine sand, forest soil and decomposed animal manure while the length of planting materials are measured according to the number of nodes such as 2,3,4 and 5 nodes. Among the parameters observed are leaf number, leaf fresh and dry weight, root length and, root to shoot ratio. Result shows that better result comes from the four node cuttings with a mixture media of forest soil.

2.2.3 Stem Spray Method

A study done by Andreu, L.G.I (2021) identified the effect of salicylic acid on both *in vitro* and in *vivo* propagation methods. The plantlets with 10 cm in length were planted in medium-sized pots containing peat moss and agrolite substrate. For *in vivo* treatment, the plantlets are sprayed at the nodal segments and leaves with different concentrations of salicylic acid (0,0.25,0.5,1 μmol of SA) in one week interval for four weeks. The result clarified that salicylic acid produces a significant impact on stem length and stem diameter and increased the root length. Therefore, the study spray method could also affect the growth and development of plants and is considered applicable for vegetative propagation of vanilla cuttings in the present study.

Chapter 3: Materials and Methods

3.1 Location of Experiment

This study was conducted at the Plant Research Centre, UNIMAS (PPT). The experiment officially started on 15th April 2022 when the plant materials were first collected. The initial objective is to study the shoot and root formation and development of *Vanilla planifolia* under different treatments.

3.2 Collection and preparation of plant materials

The vanilla cuttings were purchased from a local farmer in a nursery at Batu Kawa, Kuching. Approximately, 130 cuttings are obtained after the educational visit to the nursery (Figure 3). After that, the cuttings are subsequently processed in PPT. The vines were cut into different sizes according to the number of nodes, specifically 3,4 and 5 nodes and the leaves were completely removed. Then, the vines or cuttings were sterilized using Dettol solution to remove any contaminant and avoid fungal or bacteria infection. Next, vanilla cuttings were left to dry and thus directly planted in the cocopeat-sand medium.

Figure 3: The vanilla vines are obtained from the nursery in Batu Kawa, Kuching.

3.3 Preparation of media

A mixture of 1:1 ratio cocopeat and sand is chosen as the growing medium. The growing medium is prepared earlier before the cuttings are processed. Sandeep, K. et al. (2018) suggested the medium combination of cocopeat, sand and vermicompost is the best potting soil for plants due to the maximum plant height and root length. However, due to cost and time issues, we decided to proceed with the application of cocopeat mixed with sand as the closest best media.

3.4 Experimental Design

Experiment was conducted in Completely Randomized Design (CRD) using thirteen cuttings per treatment.

3.5 Preparation of Treatment

The treatment applied for *in vivo* propagation of *Vanilla planifolia* includes different concentrations of BAP, GA3 and NAA+BAP. All treatments were prepared by dissolving 5 mL, 10 mL and 15 mL of each hormone into a can of sprayer, respectively (Figure 4). Initial volume of distilled water must be less than 50 mL at first pour and to be filled upto 100 mL later to avoid inaccurate hormone concentration. The preparation was done in the laboratory to avoid contamination from the environment. In this study, 13 cuttings were subjected to hormones (BAP, GA3 and NAA+BAP) at different concentrations (5,10,15 mg.L), respectively. Therefore, overall 130 cuttings were prepared and used in this study.

Figure 4: Treatments with different types and concentrations of hormones are prepared in the sprayers, respectively.

3.6 Treatments with Different Types and Concentrations of Hormones

Treatments are as listed below.

1) T0 = control
2) T1 = 5 mg/l BAP
3) T2 = 10 mg/l BAP
4) T3 = 15 mg/l BAP
5) T4 = 5 mg/l GA3
6) T5 = 10 mg/l GA3
7) T6 = 15 mg/l GA3
8) T7 = 5 mg/l NAA + 5 mg/l BAP
9) T8 = 5 mg/l NAA + 10 mg/l BAP
10) T9 = 5 mg/l NAA + 15 mg/l BAP

3.7 Data collection

Observations are done after 30 days of planting. Among the parameters observed in this study are as follows.

a) Number of Roots: The number of roots were recorded 30 days after the first day planting by counting the number of roots emerged and elongated within the media. Mean number of roots was obtained for each treatment.
b) Length of Roots: The length of roots were measured using a ruler after the cuttings were removed from the media temporarily for root measurement during the 30^{th} day. Mean length of roots was obtained for each treatment.

c) Diameter of Cuttings: The data was measured using Digital Vernier Callipers to obtain the value for the cutting's diameter. Mean diameter of cutting was obtained for each treatment.

d) Colour of Root: The data for colour of root was collected to identify whether the cuttings can produce healthy roots or not. Colours observed are white and brown and the data recorded as 1 and 0. 1 scored for a healthy white root and 0 for no formation of white root.

e) Rate of Survival: The data was collected by observing the number of cuttings survived after 30 days over all initial total cuttings per treatment.

$$Rate\ of\ Survival: \frac{Total\ Cutting\ Survived}{Initial\ Total\ Cutting} x100\%$$

3.8 Statistical analysis

The mean values recorded were analyzed using one-way analysis of variance (ANOVA) to compare the mean values via Duncan's Multiple Range Test at 5% significance level using IBM SPSS Statistics.

Chapter 4: Results

The experiment was conducted for approximately one months (30 days). Final data collection was recorded on the 30th day. Among the parameters observed (Table 1) were the survival rate (%), shoot and root production (%), mean number of shoots and roots per explant, and shoot and root length (cm). Below is the overall result for all parameters observed and the analysis obtained from the SPSS.

Table 1: Effects of various treatments on all parameters observed on the 30th day of planting

	Number of Root	Length of root	Diameter cuttings	White Root formation (%)	Brown Root Formation (%)	Survival rate
Treatment	**Mean ± Standard Error**					
Control	0.77 ± 0.20a	2.67 ± 1.00ab	7.16 ± 1.42a	61.54 ± 0.14a	7.69 ± 0.77a	69.23 ± 0.13a
5 mg /L BAP	0.77 ± 0.20a	2.16 ± 0.83ab	6.72 ± 1.56a	53.85 ± 0.14a	7.69 ± 0.77a	61.54 ± 0.14a
10 mg /L BAP	0.77 ± 0.20a	4.52 ± 1.44b	6.82 ± 1.58a	53.85 ± 0.14a	7.69 ± 0.77a	61.54 ± 0.14a
15 mg /L BAP	0.46 ± 0.24a	0.81 ± 0.44a	5.42 ± 1.43a	30.77 ±0.13a	0.00 ± 0.00a	69.23 ± 0.13a
5 mg /L GA3	0.31 ± 0.17a	1.04 ± 0.63a	5.53 ± 1.52a	23.08 ± 0.12a	0.00 ± 0.00a	46.15 ± 0.14a
10 mg /L GA3	0.46 ± 0.18a	1.88 ± 0.9ab	3.94 ± 1.45a	38.46 ±0.14a	0.00 ± 0.00a	38.46 ± 0.14a
15 mg /L GA3	0.54 ± 0.18a	3.54 ± 1.21ab	5.41 ± 1.47a	38.46 ± 0.14a	0.00 ± 0.00a	53.85 ± 0.14a
5 mg/L NAA + 5 mg /L BAP	0.69 ± 0.26a	2.25 ± 0.98ab	5.45 ± 1.48a	46.15 ±0.14a	7.69 ± 0.77a	53.85 ± 0.14a
5 mg/L NAA + 10 mg /L BAP	0.77 ± 0.26a	2.50 ± 0.90ab	8.16 ± 1.32a	53.85 ± 0.14a	7.69 ± 0.77a	76.92 ± 0.12a
5 mg/L NAA + 15 mg /L BAP	0.46 ± 0.18a	2.27 ± 0.97ab	4.45 ± 1.40a	38.46 ± 0.14a	7.69 ± 0.77a	46.15 ± 0.14a

Mean values ± SE analysed using one-way analysis of variance (ANOVA) via Duncan Post Hoc at p=0.05, n=13