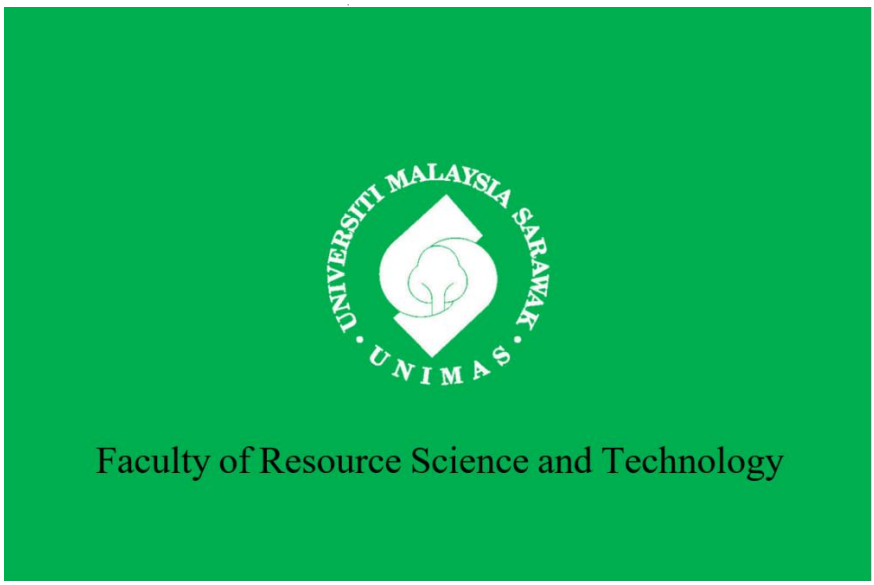

Vegetative propagation of *Vanilla borneensis in vivo*

Nur Shamimi Izzati Binti Mohd Shukri (72560)

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

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Nur Shamimi Izzati Binti Mohd Shukri

A thesis submitted in partial fulfillment of the Requirement of The Degree Bachelor of Science with Honours
(Plant Resource Science and Management)

SUPERVISOR : DR HASHIMAH BINTI ELIAS

Programme of Plant Resource Science and Management
Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2022

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Vegetative propagation of *Vanilla borneensis in vivo*

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ABSTRACT

Vanilla borneensis is a monopodial climbing orchid that is critically endangered in the areas where it is indigenous to Southeast Asia and commonly found in particular parts of the Malay Peninsula and Peninsular Thailand. Generally, the seeds require a favourable environment or ideal climate for germination. *V. borneensi*s takes about 3-5 years from juvenile (seed germination) to develop into the reproduction phase (vanilla pod production). Considering the issues and potential as an alternative to the other commercial *Vanilla* spp., a study on the *in vivo* propagation via stem cutting was conducted to ensure the sustainability of plant resources and provide sufficient planting material for further studies. In the present study, the stem of *V. borneensis* was sectioned into approximately 30-50 cm in length or 2-5 nodes per cutting. The explants were properly washed and soaked in disinfectant before being planted in the growing medium, 1:1 cocopeat and sand. After 2 weeks of planting, different types (BAP, NAA, GA3) and concentrations (0,5,10,15 mg/L) of plant growth regulators were applied by spraying the explants weekly. Observations were recorded and analysed on the mean percentage of root formation before (B) and after (A) sprayed with treatments, number of roots produced per cutting, root length and percentage of white root production. In this study, treatment with 10 mg/L BAP was recommended for propagation of *V. borneensis in vivo* which gave better results for all parameters observed. However, further studies should be done by considering the other factors including a longer time frame for observations, selecting older explant sources, increasing the concentrations of plant growth regulators and growing the cuttings in different orientations to validate the findings besides providing sufficient planting materials and primary data for further studies on the propagation of *V. borneensis in vivo*.

Key words: Orchidaceae, plant growth regulators, BAP, NAA, GA3.

ABSTRAK

Vanilla borneensis merupakan orkid memanjat monopodial yang sangat terancam di mana ia berasal dari Asia Tenggara dan biasa ditemui di bahagian tertentu di Semenanjung Tanah Melayu dan Semenanjung Thailand. Secara amnya, biji benih memerlukan persekitaran yang baik atau iklim yang sesuai untuk percambahan. V borneensis mengambil masa kira-kira 3-5 tahun dari juvana (percambahan biji) untuk berkembang menjadi fasa pembiakan (pengeluaran pod vanila). Mengambil kira isu dan potensi sebagai alternatif kepada Vanila spp. komersial yang lain, satu kajian mengenai pembiakan in vivo melalui keratan batang telah dijalankan untuk memastikan kemampanan sumber tumbuhan dan menyediakan bahan tanaman yang mencukupi untuk kajian lanjut. Dalam kajian ini, batang V. borneensis telah dibahagikan kepada kira-kira 30-50 cm panjang atau 2-5 nodal setiap keratan. Eksplan dicuci dengan betul dan direndam dalam disinfeksi sebelum ditanam dalam medium penanaman, cocopeat 1:1 dan pasir. Selepas 2 minggu penanaman, pelbagai jenis (BAP, NAA, GA3) dan kepekatan (0,5,10,15 mg/L) pengawalatur pertumbuhan telah digunakan dengan menyembur eksplan setiap minggu. Pemerhatian telah direkodkan dan dianalisis pada purata peratusan pembentukan akar sebelum (B) dan selepas (A) disembur dengan rawatan, bilangan akar yang dihasilkan setiap keratan, panjang akar dan peratusan pengeluaran akar putih. Dalam kajian ini, rawatan 10 mg/L BAP disyorkan untuk pembiakan V. borneensis in vivo yang memberikan hasil yang lebih baik untuk semua parameter yang diperhatikan. Walau bagaimanapun, kajian lanjut perlu dilakukan dengan mempertimbangkan faktor-faktor lain termasuk jangka masa yang lebih lama untuk pemerhatian, memilih sumber eksplan yang lebih matang, meningkatkan kepekatan pengawalatur pertumbuhan dan menanam keratan dalam orientasi yang berbeza untuk mengesahkan penemuan selain menyediakan bahan penanaman dan data primer yang mencukupi untuk kajian lanjut tentang pembiakan V. borneensis vivo.

***Kata kunci**: Orchidaceae, pengawalatur pertumbuhan, BAP, NAA, GA3.*

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Abbreviation	Meaning
UNIMAS	University Malaysia Sarawak
BAP	6-Benzylaminopurine
NAA	Naphthyl acetic acid
GA3	Gibberellic acid
ANOVA	One-way analysis of variance
DMRT	Duncan's Multiple Range Test
NaOH	Sodium hydroxide

CHAPTER 1 : INTRODUCTION

Vanilla is a tropical vine orchid that requires high humidity with a yearly rainfall of 1500 mm to 3000 mm (Kerala Agriculture University, 2016). Its native range includes Central America, southern Mexico, the West Indies, and northern South America (Njoroge et al., 2005). Meanwhile, Madagascar, the Malagasy Republic, Indonesia, the Comoros, and Uganda are the primary vanilla producing countries. Vanilla is a climbing perennial orchid with sessile leaves and succulent green stems that generate aerial roots at the nodes. Generally, the seeds require a favourable environment or ideal climate for germination. It takes about 3-5 years from the juvenile to the reproduction phase, from seed germination to the production of pods. Thus, propagation by stem cuttings which is much easier and produces yields earlier is preferable. By stem cuttings, the clones produced are genetically identical to the mother plants.

There are about 180 known Vanilla species worldwide and the popular commercial species are *V. planifolia, V. tahitensis* and *V. pompona* that could produce vanillin from the cured vanilla beans. This valuable compound is extracted and used as fragrance and flavours in food, pharmaceutical, cosmetic and perfumery industries and also for handicraft products. In Malaysia, there are ten wild Vanilla species that have been reported including *V. borneensis* which has been categorised as critically endangered due to habitat loss and unsustainable exploitation. Moreover, the issues related to climate change also influenced the propagation of the species in its natural habitat. A propagation method by cuttings offers practical action to provide planting materials for the recovery programme or reintroduction efforts for the conservation of the species. *Vanilla* sp. is difficult to cultivate and requires significant principle, so either by hormone spraying or sterilisation technique, it is a decent solution to the issue and gives rise to the propagation of vanilla in a large quantity.

1.1 Problem Statement

Vanilla borneensis is a wild species that belongs to the subfamily Vanilloideae. It is a herbaceous plant that is abundantly found in tropical and subtropical regions. There is still a lack of studies focusing on this native species. The species was observed to possess desirable traits as a promising genetic resource to replace other commercial Vanilla species. Considering the potential benefits, a study on the propagation of this species was conducted to ensure the sustainability of plant resources and provide sufficient planting material for further studies.

1.2 Objectives

The objectives are:

i. To investigate the effects of different treatments on the propagation of *Vanilla borneensis in vivo*.

ii. To determine the best treatment (types and concentrations) for the growth and development of *Vanilla borneensis*.

CHAPTER 2 : LITERATURE REVIEW

2.1 Family Orchidaceae

The Orchidaceae family, also known as orchids, is the largest flowering plant kingdom and is notable for its exotic and intricate beauty of flowers, as well as their pleasant fragrance (Arditti, 2008). It has many species in the range of 20,000 to 35,000 which are classified into five subfamilies including Apostasioideae, Vanilloideae, Cypripedioideae, Orchidoideae, and Epidendroideae. They invaded and occupied almost every country excluding the true desert and frozen Antarctica. The distribution of this perennial monocotyledonous plant is not uniform but skewed markedly towards the tropics. They are easily distinguished from other plants by their unique characteristics like having bilateral symmetry of the flower (zygomorphism), many resupinate flowers, a nearly always highly modified petal (labellum), fused stamens and carpels, and extremely small seeds.

According to Go *et al.* (2020), they comprise up to 10% of all flowering plants and include approximately 1026 species from 155 genera locally. There are approximately 3000 orchid species recorded throughout Malaysia (Ong *et al.*, 2017; Juiling *et al.*, 2020; Forest Department Sarawak, 2021). It offers a significant contribution not only to Malaysia's floriculture industry but others as well, with a high market demand and potential for health benefits. Species that belong to the genera Anoctochilus and Dendrobium have been identified to have medicinal properties and are prevalently used in traditional Chinese folk remedies (Bijaya, 2013). The orchid industry has been introduced in Malaysia since the 1960s and has become popular and developed recently. New orchid cultivars should be developed on a constant basis to meet the market demand and satisfy the customers' needs. Orchids are now a multimillion-dollar business,

potentially arising from the potted plant trade and floriculture cut flowers (Roberts & Dixon, 2008; Chugh *et al*., 2009).

2.1.1 Subfamily Vanilloideae

There are about 110 species in the Vanilla genus, which vary from tropical to subtropical regions (Cameron, 2011). According to Bory *et al*. (2007), the most diverse *Vanilla* species have been found in tropical America, followed by Southeast Asia and New Guinea. The genus Vanilla has alternating leaves along the stem and can reach heights of up to 35 metres. Vanilla leaves are usually elongated in shape and dark green in colour. Nevertheless, some species have scaly leaves or are utterly leafless. The aerial roots sprout from each node, enabling the plant to climb (van Noort, 2018). In Malaysia, *Vanilla* species are vulnerable to various threats along with an insufficiency of taxonomic data and modification, forest fragmentation, environmental disaster, and the natural leaves abscission occurrence (Raffia *et al*., 2016).

2.1.1.1 *Vanilla borneensis*

Vanilla borneensis Rolfe is an endemic and critically endangered monopodial climbing orchid that is native to Assam, India. Its worldwide distribution ranges from particular parts of Malaya, Thailand and Borneo. This species is indigenous to Southeast Asia and can be found in particular parts of the Malay Peninsula and Peninsular Thailand, where it grows in dry evergreen mountainous forests at an elevation ranging from sea level up to 1000metres. The species had first been clarified in Malaya but now it has been registered in Thailand, Assam's Mikir Hills (Karbi Anglong), and Golaghat District's Nambar Reserve Forest in Northeast India (Borthakur & Hajra, 1976).

Vanilla borneensis Rolfe has also been named as *Vanilla pilifera* Holttum in which both of these species are equivalent to each other (Baruah, 1998). This species has a

narrow type of leave as shown in Figure 1 and short inflorescences (Soto & Cribb, 2013). The sepals are light green and white at the base, whereas the petals are greenish white in colour, white at the base and the midribs are light green. The lip petal mid-lobe is membraneaceus with fine light purple lines on the lateral lobes, hairs on the mid-lobe and pale purple at the base. The median region of the lip petal is densely covered with lilac, back pointed bristles at the posterior part and creamy lilac curved bristles at the interior part (Bijmoer *et al*., 2021).

2.2 Propagation by stem cuttings

Vanilla sp. is often propagated through stem cuttings approximately 90 to 100 cm in length with at least 12 nodes taken from the healthy vigorous part of the vine. Healthy vines are preferred and recommended for planting (Carlos & Balakrishnan, 1991). Generally, 2 to 3 leaves are stripped away from the bottom before being placed into the humid layer and mulch. However, a study on propagation of Vanilla sp. via stem cuttings in situ at Kerala Agriculture University (2016) clarified that 60 cm vines are the recommended cutting size for root induction. Besides, the longer cuttings produce fruits earlier than the shorter cuttings. Purseglove (1973) also described that unrooted cuttings of 1 m in length could be used for propagation in direct field planting. Planting of rooted cuttings is practical for plants that require significant care and a desirable growth environment (Verheij, 2004). Organic manures could be applied to the finest layer of the soil from May until June. Meanwhile, NPK in 2 to 3 partitions together with compost could be used on the finest layer of the soil from June until September when adequate water is applied. The genus of Vanilla reacted to foliar feeding in almost the same way that other orchids use it.

2.3 Factors affecting plant propagation through stem cutting

2.3.1 Plant growth regulators

The plant growth regulators that will be applied in this study for the vegetative propagation of Vanilla borneensis are 6-Benzylaminopurine (BAP), naphthyl acetic acid (NAA) and gibberellic acid (GA3). In this study, different concentrations of each of these hormones are used and compared. BAP is the most widely used adenine-based cytokinin and is often used as a supplement in plant growth media. It influences plant growth and development. Moeini and Sanavy (2003) used different concentrations of NAA and BAP for root induction and found that application of BAP and NAA decreased shooting and rooting of single nodes. Generally, auxins, such as naphthaleneacetic acid (NAA), enhance orchid seed germination and seedling growth (Hew & Clifford,1993). 6-Benzylaminopurine, benzyl adenine, BAP or BA is a first generation cytokinin plant growth regulator influencing plant growth and development, setting blossoms and stimulating fruit richness by stimulating cell division. Thus, due to the results obtained from previous studies, different concentrations of plant growth regulators (BAP, NAA and GA3) were used in this study, such as: 5 mg/L, 10 mg/L and 15 mg/L.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Study site

The study was carried out in the greenhouse at the Plant Research Centre, East Campus of UNIMAS starting from March 2022 until May 2022 (Figure 1.0). The study focused on the responses of stem cuttings towards the growth and development of *Vanilla borneensis* after being treated with different treatments (types and concentrations) of plant growth regulators (BAP, NAA and GA3). The cuttings were grown in shaded areas according to environmental conditions suitable for *Vanilla* species.

Figure 1.0 The cuttings were placed under a shaded area in the greenhouse at Plant Research Centre, UNIMAS

3.2 Collection and preparation of cuttings

Healthy cuttings of *V. borneensis* were collected in the forest at Serian, Kuching, as depicted in Figure 2.0 (a). The cuttings were subsequently sectioned into small pieces in the range of 30-50 cm in length (2 to 5 nodes), as the planting materials or explants were used in this experiment whereby the leaves on the cuttings were totally removed as shown in Figure 2.0 (b). The explants were washed properly and soaked in disinfectant for 15 minutes to remove

all the dirt and contaminants. Then, the explants were dried and stored in a cool, shaded area for 2-3 days to prevent moisture loss and improve rooting. Next, the explants were planted in the polybags containing growing media that were already prepared. The growth of the cuttings before being sprayed (treated with hormones) was monitored daily. From the observations, some of the cuttings were dried. Thus, the replacement of the dried cuttings was done after 1 week .

(a) (b)

Figure 2.0 (a) *V. borneensis* are collected in the forest at Serian, Kuching and (b) sectioned into pieces (2 to 5 nodes per cutting) as the planting materials.

3.3 Preparation of growing medium

In this experiment, the growing medium used was a mixture of coco peat and sand in a ratio of 1:1. Preparation of the growing medium was done 3 days before the cuttings were collected. At first, the coco peat was soaked in the Styrofoam box filled with water for 2 days before being mixed with sand (Figure 3.0). The growing medium containing the mixture of

coco peat and sand (1:1) was filled into the polybags. A total of 180 polybags were used in this study.

Figure 3.0 Coco peat was soaked for 2 days in Styrofoam box before being mixed with sand

3.4 Preparation of hormone

The treatments applied for the vegetative propagation of *Vanilla borneensis* include the application of different types of plant growth regulators such as BAP, NAA and GA3 at different concentrations. Preparation of hormones was done in the laboratory to avoid any contamination from outside sources. All of the treatments were prepared by dissolving the hormones in powder form into distilled water following the proposed concentrations. The stock solution of each plant growth regulator is generally dissolved first by a few drops of solvents such as 1.0 M NaOH or 1.0 M KOH and then subsequently followed by the addition of distilled water. The beaker containing the hormone solution was then wrapped in aluminium foil and placed in the refrigerator at 2°C. To prepare the BAP hormone solution, 50 mg of BAP powder

was weighed and put into a 100 ml beaker. A small amount of 1N NaOH was also added drop by drop and the beaker was stirred until the powder was completely dissolved.

The hormone solution is then diluted with distilled water. The treatments were made by combining the stock solution and distilled water in the appropriate concentration (5 mg/L, 10 mg/L and 15 mg/L). It was necessary to stir the solution while adding water. Figure 4 showed that the hormones prepared were poured into the sprayer depending on its type (BAP, NAA, GA3) and concentration(5 mg/L, 10 mg/L and 15 mg/L) of hormones. The hormones were sprayed (2 to 3 mL) weekly starting from week 2 onwards. The same procedure was repeated for other types of hormones (NAA and GA3). PGRs (BAP, NAA and GA3) are typically prepared at a concentration of 1 mg/L and stored at -2°C. Occasionally, the concentrated stock hormone solutions of some less soluble PGRs may precipitate during cold storage (Figure 5.0). Therefore, it must be heated to re-dissolve the compound before use.

Figure 4.0 The hormones prepared were poured into sprayers

Figure 5.0 The stock hormones kept in the refrigerator may precipitate during cold storage

Table 1.1 Treatments applied in propagation of *Vanilla borneensis* in vivo

No.	Treatments	Number of Cuttings
1.	0 mg/l (control)	18
2.	5 mg/l + BAP	18
3.	10 mg/l + BAP	18
4.	15 mg/l + BAP	18
5.	5 mg/l + NAA	18
6.	10 mg/l + NAA	18
7.	15 mg/l + NAA	18
8.	5 mg/l + GA3	18
9.	10 mg/l + GA3	18
10.	15 mg/l + GA3	18
	Total	**180**

3.5 Experimental design and Statistical analysis

A total of 180 explants or cuttings were used in each study whereby each treatment consisted of 18 explants or cuttings. The study will be conducted as a factorial experiment (3 x 3) in a complete randomized design (CRD). Data was analyzed using dependent samples T-test for the percentage of root formation and one-way ANOVA analysis via Tukey's HSD Post Hoc at $P \leq 0.05$ for the number of roots produced per explant, length of roots and the percentage of white and brown root formation.

CHAPTER 4 : RESULTS

4.1 The effect of different treatments towards the growth and development of *V. borneensis* via stem cutting

This study focused on the responses of stem cuttings after being treated with different types and concentrations of plant growth regulators (BAP, NAA and GA3). The data for the growth and development of *Vanilla borneensis in vivo* were recorded and analysed after being sprayed weekly onwards for 32 days. Generally, the root formation was observed in week 2 (Figure 6.0) before the cuttings were sprayed by plant growth regulators. In this experiment, the cuttings were classified into different sizes and then planted in the growing medium. In week 2, the cuttings were sprayed with different types and concentrations of plant growth regulators (Table 4.1). Among the parameters recorded were the mean percentage of root formation, the mean number of roots produced per explant, the mean length of roots and the mean percentage of white and brown root formation as presented in Table 4.2, 4.3 and 4.4, respectively.

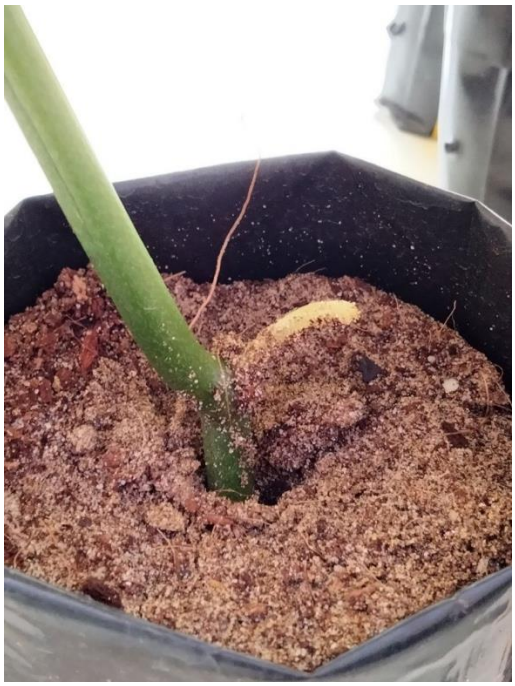

Figure 6.0 The root formation was observed in week 2 before the hormones sprayed on the cuttings