FLORAL DEVELOPMENT PATTERN AND *In Vitro* PROPAGATION OF *Paraphalaenopsis labukensis* P.S. SHIM, A. LAMB & C.L. CHAN

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotation, summaries and references, which have been duly acknowledged.

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ABSTRACT

Paraphalaenopsis labukensis is a monopodial epiphytic species that only can be found in Sabah. *P. labukensis* orchids have unique characteristics in which it has a long floral lifespan as compared to other orchid species. The flower developmental pattern of *P. labukensis* greatly influenced the capsule formation and seed maturation. Through morphological studies and flower development stages, the maturity period of the orchid pod can be observed. The present research was conducted to record the initiation of flower morphological and development patterns as well as the effect of growth hormones on asymbiotic seed germination and their subsequent development into plantlets. By identifying the duration of the whole flowering developmental process, this will aid in the production of capsules to attain a reliable and adequate seed source for *in vitro* seed germination. The flowering stages were observed based on the length of inflorescence, diameter and length of bud, a number of flowers produced, and length of capsule formed. As a result, overall there were twelve stages of floral development involved. In this study, 120 days after pollination (DAP) capsule was selected as the most suitable capsule age for germination as it had reached its maturation period. After 90 days of culture, the highest germination percentage was achieved in Knudson C (KC) medium, with 98.78±0.89% followed by Murashige & Skoog (MS) and Vacin & Went (VW) medium (92.80±3.26%, 84.17±1.68%) respectively. Medium containing banana homogenate (BH) (5 mg/L) was the best complex additive for protocorm proliferation with 15.75±0.96% compared to coconut water. In addition, treatment with single PGRs has shown that 0.5 mg/L NAA has the highest percentage of proliferation (17.25±0.96%) followed by 1.5 mg/L NAA (16.50±0.50%). Meanwhile, for shoot induction, the highest rate recorded was in 1.0 mg/L NAA+0.5 mg/L BAP (34.75±0.56%). Combination of 1.0 mg/L NAA+0.5 mg/L BAP has recorded the highest percentage of root induction with 18.50±0.57%. This study showed that asymbiotic seed germination and seedlings development of *P. labukensis* is an efficient technique and can be applied as a conservation tool to protect the local orchid species.

ABSTRAK

CORAK PERKEMBANGAN BUNGA DAN PROPAGASI SECARA IN VITRO Paraphalaenopsis labukensis P.S SHIM, A. LAMB & C.L CHAN

Paraphalaenopsis labukensis merupakan salah satu orkid monopodial bersifat epifit yang hanya dapat dijumpai di Sabah. Antara ciri-ciri yang unik bagi spesis orkid ini ialah proses pembungaan yang panjang berbanding spesis orkid lain. Proses pembungaan P. labukensis banyak mempengaruhi fasa pembentukan pod dan juga kematangan biji benih. Melalui kajian morfologi dan perkembangan bunga, tempoh kematangan pod dan biji benih dapat dikawal. Kajian ini telah dijalankan bagi merekodkan morfologi dan kitaran perkembangan bunga serta mengkaji kesan penggunaan media asas, komplek aditif dan pengawalatur tumbuhan ke atas percambahan biji benih, proliferasi serta perkembangan protokom P. labukensis. Dengan mengenalpasti tempoh keseluruhan bagi proses pembungaan P. labukensis, maka secara tidak langsung dapat membantu di dalam proses pembentukan kapsul serta bijih benih yang mencukupi bagi proses propagasi in vitro. Secara keseluruhan, terdapat 12 peringkat perkembangan bunga. Walau bagaimanapun, terdapat lima peringkat utama perkembangan bunga iaitu bermula dengan pertumbuhan tunas, perkembangan tunas, perkembangan infloresen bunga dan seterusnya pembentukan kapsul. Kapsul berumur 120 hari telah dipilih sebagai sumber eksplan yang sesuai setelah mencapai tahap kematangan yang sesuai. Pemerhatian selepas 90 hari pengkulturan mendapati peratus percambahan biji benih P. labukensis mencapai peratusan tertinggi di dalam media asas Knudson C (KC) *(98.78±0.89%) diikuti media asas* Murashige & Skoog (MS) *dan* Vacin & Went (VW) *masing-masing menunjukkan peratusan sebanyak 92.80±3.26% dan 84.17±1.68%. 5g/l (w/v) homogenate pisang merupakan media proliferasi terbaik berbanding media air kelapa sebanyak 15.75±0.96%. Selain itu, kesan pengawalatur tumbuhan tunggal juga turut memberikan peratusan proliferasi tertinggi sebanyak 17.25±0.96% di dalam media 0.5mg/l NAA diikuti dengan 1.5mg/l NAA (16.50±0.50%). Manakala, peratusan tertinggi bagi pembentukan daun telah dicapai di dalam media kombinasi pengawalatur tumbuhan 1.0mg/L NAA+0.5mg/L BAP (34.75±0.56%) Selain itu, kombinasi tersebut juga turut memberikan peratusan tertinggi bagi pembentukan*

akar P. labukensis (18.50±0.57%). *Kajian ini menunjukkan bahawa teknik propagasi secara asimbiotik adalah teknik yang efisien dan dapat diaplikasikan sebagai medium konservasi bagi melindungi spesis-spesis orkid yang terancam.*

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ANOVA	-	Analysis of Variance
BAP	-	Benzylaminopurine
BH	-	Banana homogenate
CW	-	Coconut water
Hr	-	Hour
KC	-	Knudson C medium
mg/l	-	Miligram per litre
mm	-	Milimeter
MS	-	Murashige and Skoog medium
NAA	-	Napthalene acetic acid
PGR	-	Plant growth regulators
pH	-	Power of hydrogens
v/v	-	Volume per volume
VW	-	Vacin and Went medium
w/v	-	Weight per volume
DOC	-	Days of culture
DAP	-	Days after pollination

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CHAPTER 1

INTRODUCTION

The Orchidaceae is one of the largest families of the flowering plant which is economically valuable in the floriculture industry as cut flowers and potted plants (Utami & Hariyanto, 2019). The island of Borneo is one of the centres of orchid diversity (Hassler & Rheinheimer, 2020). The estimated number of orchid species in Borneo varied widely. Species richness of orchids in Borneo has been partially related by the presence of Mount Kinabalu, the highest mountain in the Malay Archipelago (Suzika *et al.,* 2020). Orchids are among the most flowering plants in the world and became favourites in horticulture and contain many genera (Dutta *et al.,* 2011). It is also among the most widely distributed. Variations in sizes of orchids were from a few 3-4 mm to several meters, while for its flowers, they range from 2-3 mm to 15-20 cm (Arditti, 1967). The beauty of orchid flowers, variety in its fragrance, brilliance in colours and attractive habitat has aroused the highest admiration among the people throughout the world (Dutta *et al.,* 2011). There has been lots of orchid species which are classified as endangered species such as *R. bella, P. rothschildianum, P. lowii, P. laycockii, P. gigantea, P. cornucervi, B. alatum, D. tomaniense, R. odobenus* (Lamb, 1991 & IUCN, 2020).

Paraphalaenopsis labukensis, is one of the endangered and endemic orchid in Sabah (Chan *et al.,* 1994) also known as the most spectacular pendant epiphytic orchid in Kinabalu Park. This particular plant grow suspended from small trees three to five metres above the ground or twelve to twenty metres up on the trunks and branches of larger trees (Wood *et al.,* 1993). The study on the lifespan of orchids as well as its flower morphological development is an interesting topic to be studied in which it involved various stages of the flower development. However, nowadays there were not much documentation on the floral development of most orchid species

and in some species, has not yet been done. The floral development stages or their flowering pattern however has a crucial role towards the *in vitro* seed germination process. However, the rich diversity and their distinctive flowers make them popular among orchid collectors and prone towards their threats such as overcollecting, habitat loss and disturbance (Paul *et al.*, 2012). To meet both local and foreign demand, orchid dealers depends mainly on ruthless collection or in other words as over-collection of orchids. As a result, many rare orchid species had become endangered. Therefore, to prevent the extinction and depletion of this species, an efficient and extensive study of orchid development and production must be carried out. Hence, to solve this problem, the asymbiotic germination technique was introduced since the conventional way of propagating orchid species are slow (Birhalawati *et al.*, 2014). Asymbiotic seed germination technique was widely used as one of the conservation tools of most orchid species. Also, this technique has been proved to be an efficient approach which that can germinate up to 100% without wasting the function of seeds (Stewart & Kane, 2006; Jaime *et al.*, 2005; Kauth *et al.*, 2006).

Until today, there is still no report on the floral developmental stages and *in* vitro germination for *P. labukensis.* Hence, in this study, the flower development pattern was investigated, and parameters involved such as length and diameter of bud and inflorescence, length of initial spike, average size of flower, length of capsule. Different capsule age of *P. labukensis* will be tested with three different basal media namely KC, MS and VW. Basal media plays a crucial role as culture medium as different species of orchid react differently to each basal media and to observe its effects on the germination of orchid seeds.

Proliferation and development of *P. labukensis* were observed by treated with complex additives and PGRs. Complex additives had long been used and supplemented in basal media to promote protocorm proliferation and development for orchid *Dendrobium* Alya Pink (Nambiar *et al.,* 2012) and *Phalaenopsis violacea* (Gnasekaran *et al.,* 2010). Two types of complex additives used in this study are banana homogenate and coconut water to investigate their effects on protocorm proliferation and development. For plant growth regulators, the effects of both single and combination hormones were tested and observed for the protocorm development. Plant hormones treated in both single and combination were reported

to enhance shoot and root induction in *Dendrobium chryseum* (Maharjan *et al.,* 2020) and in *Gastrochilus matsuran* (Kang *et al.,* 2020). Today, the application of *in vitro* propagation technique has been known as a successful method for *ex situ* conservation and reintroduction of endangered orchids.

1.1 Problem Statement

Until today, there were still no reports on the flowering development and the asymbiotic germination for *P. labukensis*. The number of flowers produced per plant only reached a maximum of five to six flowers. Hence, limited number of capsule obtained after each flowering season which could affects the *in vitro* germination for *P. labukensis*. The morphological structure of buds and flowers in *P. labukensis* has not been well identified. Scanning electron microscopy (SEM) is used to increase the accuracy of plant and flower surface of observations, as well as the development of floral organs. The images provide a more detailed view of the floral organs and the leaf surface features of the stomatal guard cells, epidermis and hairs (if present).

Also, the maturation period for capsule formation took longer time. Hence, the study on the overall pattern of flower development of this species could help to determine the most suitable capsule age to increase the germination rate of *P. labukensis.* Orchid seeds are very minute-dust like structure and they are produced in large numbers (Arditti, 1967). In nature, only 2% to 5% of seeds can be germinated in nature (Dutta *et al.,* 2011). Unfortunately, seed germination of orchid is considered difficult in nature because of a symbiosis relationship (Salifah *et al.,* 2011). Generally, orchids have very slow growth and maturation rates. Seeds of almost all orchids depend on mycorrhizal fungi to induce their germination in the wild (Chen *et al.,* 2020). In natural conditions, due to its dependency on fungus (symbiotic relationship) to be able for it to germinate, raising orchids from seeds are very difficult. Thus, the asymbiotic germination technique was introduced and applied to fulfil the demand of orchids.

1.2 Objectives

In this study, there are three main objectives to be achieved:

(a) To determine the floral development pattern and morphological features of *P. labukensis*

(b) To determine the effect of different basal media on seed germination

(c) To evaluate the effect of different concentrations of complex additives and combinations of plant growth regulators on proliferation and development