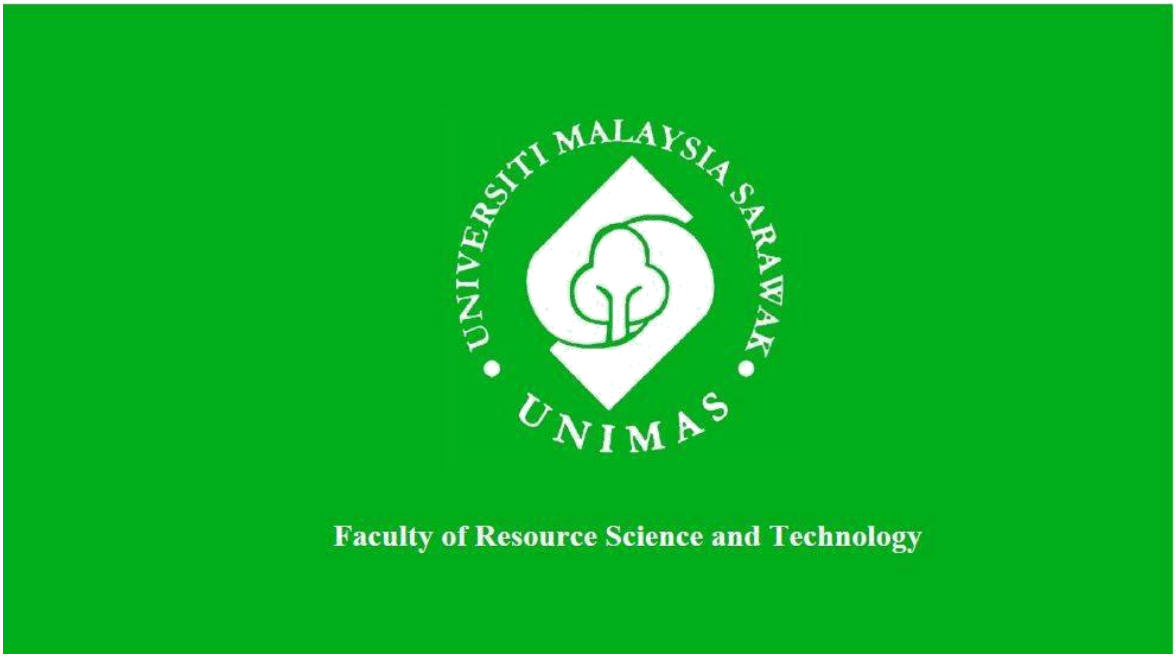

Effect of Organic Additives on *In Vitro* Seed Germination of Borneo's Endemic Orchid, *Phalaenopsis bellina* and Seedling Growth of *Vanda dearei*

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Sincerely,

Cynthia Keranti Anak Kamar

DECLARATION

I hereby declare that this thesis entitled 'Effect of Organic Additives on *In Vitro* Seed Germination of Borneo's Endemic Orchid, *Phalaenopsis bellina* and Seedling Growth of *Vanda dearei*' is my original, unaided work. I have not copied from any other sources except where the reference or acknowledgement is made clearly in the text. It has not been submitted before to any other university or tertiary institution of any degree or diploma course.

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Date Submitted Cynthia Keranti Anak Kamar

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LIST OF ABBREVIATIONS

MS	Murashige and Skoog
B5	Gamborg B-5 medium
S0	Stage 0
S1	Stage 1
S2	Stage 2
S3	Stage 3
S5	Stage 5
OA	Organic additives
PH	Potato homogenate
BP	Banana pulp
C	Control

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Effect of Organic Additives on *In Vitro* Seed Germination of Borneo's Endemic Orchid, *Phalaenopsis bellina* and Seedling Growth of *Vanda dearei*

ABSTRACT

Orchids are one of the magnificent flowers in the world. There are diverse collections of orchids in Borneo. *Phalaenopsis* and *Vanda* species are among the beautiful species of orchid that are endemic to Borneo. *Phalaenopsis bellina* and *Vanda dearei* are wild orchids in Borneo. Only a small percentage of seeds that has been released will grow into adult plants. Therefore, *in vitro* micropropagation has been applied to save and mass produce of both species. The aim of the study was to determine the effect of organic additives on *in vitro* seed germination of *Phalaenopsis bellina* and the seedling growth of *Vanda dearei* from protocorm. Seeds of *Phalaenopsis bellina* from mature capsule and protocorms of *Vanda dearei* induced from *in vitro* seed cultured were used in this study. The seeds of *Phalaenopsis bellina* were cultured on half strength of Murashige and Skoog (1/2MS) medium with 4 types of organic additives at different concentrations. Organic additives used were tomato homogenate, potato homogenate, banana pulp, and coconut water. Control treatment was prepared in the absence of organic additives. The effects of different concentrations of each organic additive on seed germination of *Phalaenopsis bellina* were examined by observing the stages of seed. There were no sign of seed germination of *Phalaenopsis bellina* with ½ MS as a basal media and supplemented with organic additives. The protocorms of *Vanda dearei* were cultured on 3 types of basal media which included Murashige and Skoog (MS), half strength of Murashige and Skoog (1/2MS), and Gamborg (B5) medium. The effects of types of basal media were investigated by observing the number of protocorms that produce leaves and roots. The result obtained that B5 medium recorded the highest percentage of protocorm producing leaves (97.25%) and roots (8.33%) followed by ½ MS and MS. As the effective basal media for seedling growth of *Vanda dearei* were B5 and ½ MS, the effect of organic additives at different concentration were investigate with these two basal media. Types of organic additives used were banana pulp and potato homogenate. The results obtained that potato homogenate with ½ MS recorded the highest percentage of protocorm producing leaves and roots.

Keywords: *Phalaenopsis bellina*, *Vanda dearei*, *in vitro*, basal media, organic additives,

Kesan Bahan Organik Tambahan dalam *In Vitro* Percambahan Benih Orkid Endemik Borneo, *Phalaenopsis bellina* dan Pertumbuhan Anak Benih *Vanda dearei*

ABSTRAK

Orkid merupakan salah satu bunga yang memukau di dunia. Terdapat pelbagai koleksi orkid di Borneo. Spesies *Phalaenopsis* dan *Vanda* adalah antara spesies orchid yang cantik dan endemik kepada Borneo. *Phalaenopsis bellina* dan *Vanda dearei* merupakan orkid liar di Borneo. Hanya peratusan kecil benih yang telah dilepaskan akan tumbuh menjadi tumbuhan dewasa. Oleh itu, *in vitro* mikropropagasi telah diaplikasikan untuk menyelamatkan dan menghasilkan kedua-dua spesies dalam jumlah yang besar. Tujuan kajian ini adalah untuk menentukan kesan bahan organik tambahan dalam *in vitro* percambahan benih *Phalaenopsis bellina* dan pertumbuhan anak benih *Vanda dearei* daripada protocorm. Benih *Phalaenopsis bellina* daripada kapsul yang matang dan protocorm *Vanda dearei* yang dikultur daripada benih digunakan dalam kajian ini. Benih *Phalaenopsis bellina* dikultur pada media asas separuh Murashige dan Skoog (1/2MS) dengan 4 jenis bahan organik tambahan yang berbeza pada kepekatan yang berbeza. Bahan organik tambahan yang digunakan adalah tomato homogenat, kentang homogenat, pulpa pisang, dan air kelapa. Rawatan kawalan disediakan tanpa kehadiran bahan organik tambahan. Kesan kepekatan yang berbeza oleh setiap bahan organik tambahan pada percambahan benih *Phalaenopsis bellina* diperiksa dengan memerhatikan peringkat pertumbuhan benih. Tiada tanda berlakunya percambahan benih *Phalaenopsis bellina* dengan ½ MS sebagai media asas dan diperkayakan dengan bahan organik tambahan. Protocorm *Vanda dearei* dikultur pada 3 jenis media asas iaitu Murashige dan Skoog (MS), separuh Murashige dan Skoog (1/2MS), dan media Gamborg (B5). Kesan jenis media asas telah dikaji dengan memerhatikan jumlah protocorm yang mengeluarkan daun dan akar. Keputusan yang diperolehi menunjukkan media B5 mencatatkan peratusan tertinggi protocorm mengeluarkan daun (97.25%) dan akar (8.33%) diikuti oleh ½ MS dan seterusnya MS. Oleh sebab media B5 dan ½ MS merupakan media asas yang berkesan untuk pertumbuhan benih *Vanda dearei*, kesan bahan organik tambahan pada kepekatan yang berbeza telah dikaji atas kedua-dua media tersebut. Jenis bahan organik tambahan yang digunakan adalah pulpa pisang dan kentang homogenat. Keputusan yang diperolehi bahawa kentang homogenat dengan ½ MS mencatatkan peratusan tertinggi protocorm mengeluarkan daun dan akar.

Kata kunci: *Phalaenopsis bellina*, *Vanda dearei*, *in vitro*, media asas, organik tambahan,

1.0 INTRODUCTION

The flora of Borneo is quite remarkable. Borneo consists of about 3000 species of tree, more than 50 species of carnivorous pitcher, and 3000 magnificent species of orchid. Some of the orchid species are rare and endangered while some of them are yet to be discovered. Orchid plants are belonging to family of Orchidacea. It is the second largest family of flowering plant after the family of sunflower, Asteracea. *Phalaenopsis* sp. and *Vanda* sp. are among the rare species of orchid in Borneo (Chairani, 2008).

Name of *Phalaenopsis* which is pronounced as fal-ee-nop-sis come from Greek word *phalaina* and it means moth-like. This species is known as Moth Orchids because the shape of its flower for certain *Phalaenopsis* sp. resembles moths in flight. As discussed in a journal by Pinaki *et al*. (2010), *Phalaenopsis* can be found throughout the tropical rainforests of South and South East Asia (Thailand, Philippines, Malaysia, and Indonesia), New Guinea, and Northern Australia. Some of the *Phalaenopsis* sp. has sweet-scented.

Name of *Vanda* which is pronounced as VAN-dah come from Sanskrit language for *Vanda tessellate* and the name *Vanda* is used for all vandaceous orchids. Most of *Vanda* sp. can be found in tropical lowlands and foothills in Thailand, Philippines, Borneo, and Kalimantan. Most of *Vanda* species has a strong scented (Jualang *et al.*, 2014).

However, *Phalaenopsis* sp. and *Vanda* sp. are both endemic species to Borneo. Both have high value in the market because of its magnificent flower and its desirable features. Some of these species are excessively collected to fulfil the market demand. Human activities such as open burning and excessive logging have led to the habitat destruction of these species (Chairani, 2008).

The exploitation of *Phalaenopsis* sp. and *Vanda* sp. with excess collection, destruction of their habitat and illegally selling to middleman has led these species to extinction. Plant

tissue culture plays important role to protect the *Phalaenopsis* sp. and *Vanda* sp. from extinction by undergoes *in vitro* seed germination and micropropagation. There are two types of *in vitro* seed germination which are symbiotic seed germination and asymbiotic seed germination.

In the symbiotic seed germination, the seeds need to be associating with mycorrhizal fungus as the fungus will provide the seeds with essential nutrients to grow. Asymbiotic seed germination is mostly used to propagate orchids. It does not require the association of fungus. The organic and inorganic nutrients need to be prepared in a form of readily available to the seed (Shenna, 2000). As discussed by Philip (2005), Lewis Knudson has successfully germinated the orchid seeds without fungus by developing the nutrient solution based on Pfeffer's solution.

Other than asymbiotic seed germination, micropropagation from protocorm also has been applied in tissue culture in order to mass produce the species of orchids. Asymbiotic seed germination and micropropagation from protocorm has been applied in germinating the rare, threatened, or endangered native orchids for conservation. Thus, *in vitro* asymbiotic seed germination and micropropagation were applied in this study. The requirements of *in vitro* seed germination and seedling growth are specific and depend on the species of orchid (Jualang *et al.*, 2014). Therefore, this study examined the influence of composition of medium such as organic additives and basal media on *in vitro* seed germination of *Phalaenopsis sp*. and the seedling growth of *Vanda sp*.

Objective

The objectives of this study were:

i. To study the effects of organic additives which are tomato homogenate, potato homogenate, banana pulp, and coconut water on *in vitro* seed germination of *Phalaenopsis bellina.*

ii. To determine the best concentration of organic additives which are tomato homogenate, potato homogenate, banana pulp, and coconut water on *in vitro* seed germination of *Phalaenopsis bellina.*

iii. To investigate the effects of different basal medium which are full strength of Murashige and Skoog (MS), half strength of Murashige and Skoog (1/2 MS), and Gamborg B5 medium on seedling growth of *Vanda dearei*.

iv. To study the effect of organic additives which are potato homogenate and banana pulp on seedling growth of *Vanda dearei*.

2.0 LITERATURE REVIEW

2.1 Orchid

Orchids has a captivating characters as it has a colourful colour, variety structure of flower and some of it has fragrant. It has been estimated 25,000 – 30,000 species of orchid in the world and 750 different genera. *Bulbophyllum, Epidendrum, Dendrobium* and *Pleurothallis* are the biggest genera of orchid. Orchid plants are members of Orchidacea which is one of the largest families in the plant kingdom (Kee *et al.,* 2011). They are monocotyledons. Orchids produced numerous numbers of dust-like seeds but not all will germinate and grow into adult plants in nature.

According to Joseph (1967), the weight of orchid seed is between 0.3 to 14 µg. These very small seeds lack of endosperm which is function as food reserves that nourish the germinating seedling. They are unable to germinate unless being infected by a symbiotic fungus that supplies the nutrient for the seed. However, symbiotic relationships do not apply to all orchid species (Noberto, 2010).

2.1.1 Orchid Growth Patterns

There are two types of growth patterns of orchids which are sympodial orchids and monopodial orchids. Sympodial orchids produce a new shoot from axillary buds of horizontal stems which is called as rhizome. This species of orchid develop thickened stems or pseudobulbs which are functioning as water storage. This allows the plant to become dormant in duration of drought. *Cymbidium*, *Dendrobium*, and *Cattley*a are among genera which belong to this category (Gregory and Cutler, 2014).

In the monopodial orchids, there are no presences of rhizome and a new leaves are continuously arise from the same apical meristem. This orchid does not produce pseudobolbs. So, they do not have dormancy period like sympodial orchid. Monopodial

orchid grow vertically with its upright stem. *Phalaenopsis* and *Vanda* are among genera which including in this category (Gregory and Cutler, 2014).

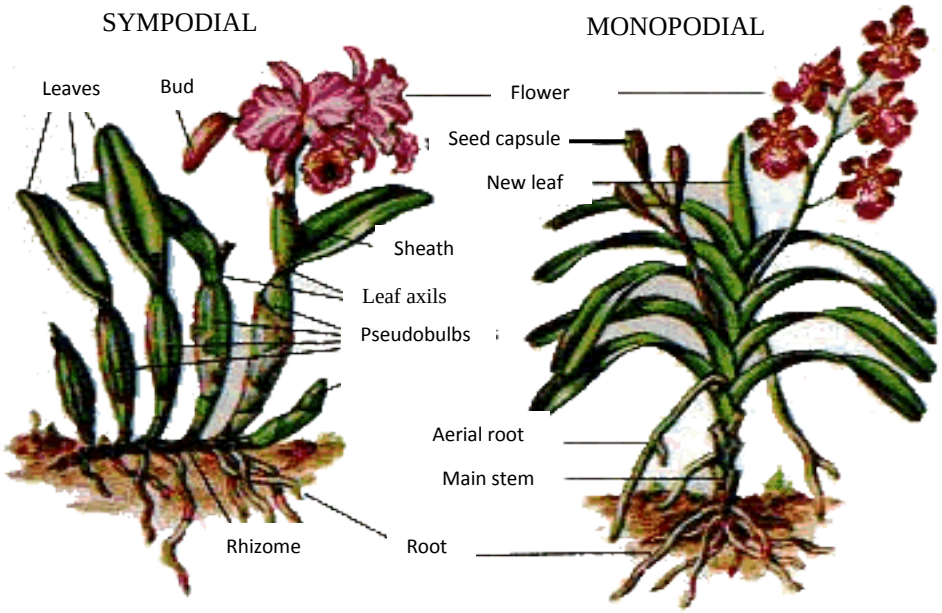

Figure 2.1: Sympodial and monopodial orchid. Retrieved from http://www.orchidcarelady.com/page/2/.

2.1.2 Orchid Growth Habit

The growth patterns of orchid are influenced by ecological habits of orchids which are terrestrial, epiphytes and lithophytes. Terrestrial orchid lives rooted in the soil. They have thin leaves and deciduous as they lives underground and lack of sunlight. *Bletilla* and *Cypripedium* belong to this group. Epiphytes orchid lives by growing on other plants but do not act as parasites. They clinging to the trees for support and absorb the moisture from the wet bark surface. Their roots are exposed to air compared to terrestrial orchid where their roots buried underground. *Phalaenopsis* and *Cattleya* are belonging to this group. Lithophytes orchid lives on the rocks (Alan, 2003). The surface of rock is suitable for attachment of seedlings.

2.1.3 Morphological of Orchids

2.1.3.1 Flower of Orchids

Orchid flower is very unique. Its flower contains three petals, three sepals and a large column which composed of stamen and pistil. The sepals are bright in colour to attract the pollinator and are unlobed. The three sepals are usually same size. The middle sepal is known as dorsal sepal whiles the other two sepals known as lateral sepal. In some species, the three sepals are different in size. As in *Paphipedilum*, the lateral sepals are fused into one and have large dorsal sepal. One of the petals is different from the other two petals as it has specialized structure known as lip or labellum. In some species, they have the same size of sepals and two petals. Whilst in some species, the two petals are larger than the sepals as in *Cattleya*. The special petal called as lip or labellum is importance in pollination. It provides a landing platform for insects to pollinate the flower. The reproductive organ of orchid is called as column. It is located in the centre of the flower. It consists of male anthers with pollinia and female pistil with ovary and stigma (Thomas, 1992).

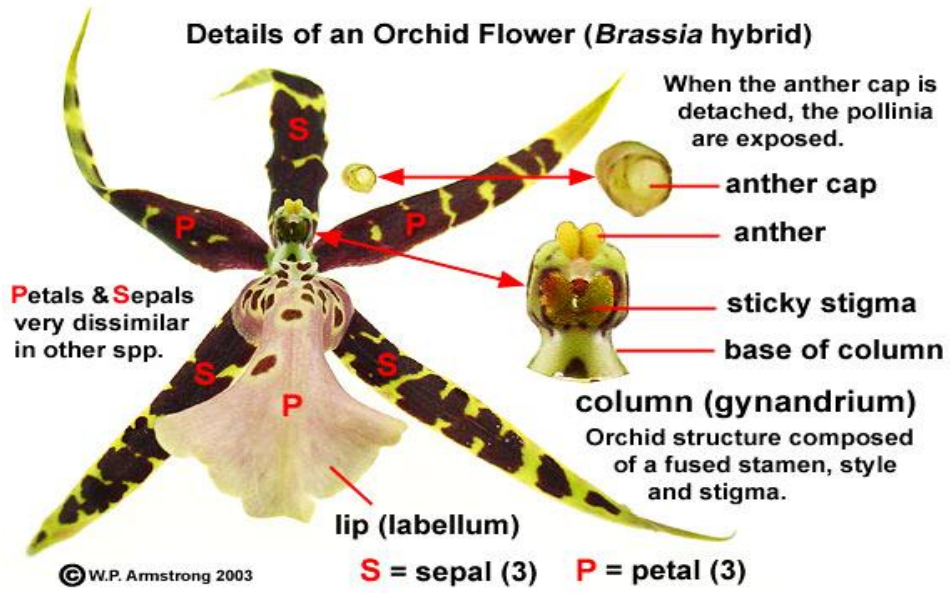

Figure 2.2: The structure of orchid flower. Retrieved from http://waynesword.palomar.edu/termfl1.htm.

2.1.3.2 Pollinating the Flower

At the top of the column, the anthers contain pollinia which are yellow colour. Mostly, the orchid contains two pollen masses. Female parts are below the male anther where the stigma located. Stigma has sticky cavity where the pollinia is located for fertilization. There is a structure called as rostellum which separates the anther and stigma to prevent self-pollination. The anther cap covers and protects the pollinia from fall. The pollinia are attached to viscidia. When the anther cap is removed by pollinator, the pollinia is exposed and transferred to sticky stigma thus fertilizing the flower.

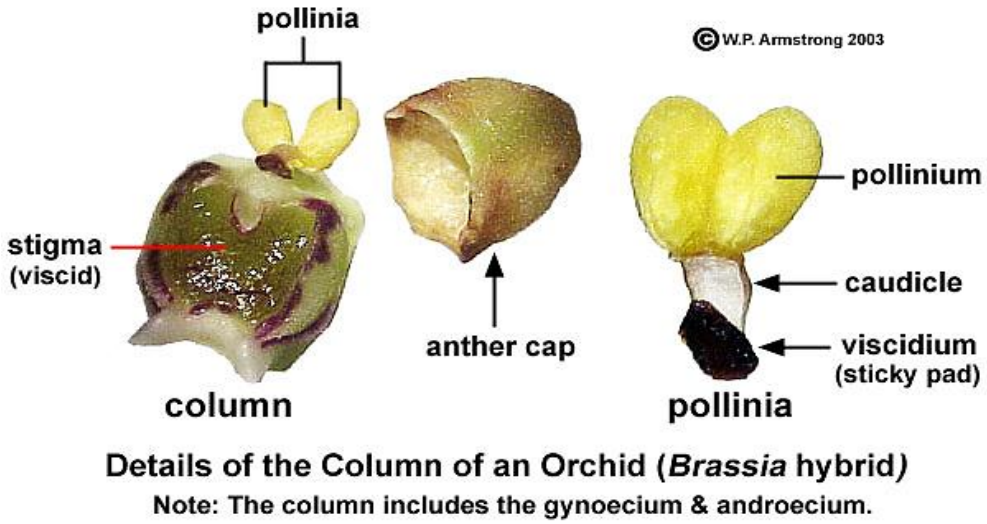

Figure 2.3: Reproductive structure of orchid. Retrieved from http://waynesword.palomar.edu/termfl1.htm.

2.1.3.3 Inflorescence of Orchid

Orchid has their own way in carrying their flower depends on genus of orchids. An inflorescence is an arrangement of flowers on axis. There are five types of inflorescence in orchid plants which are single, umbel, spike, raceme, and panicle. The major axis of an inflorescence is called as rachis. Single inflorescence is where there is only one flower on each stem. This type of inflorescence usually has large flower. Single inflorescence can be seen in *Paphiopedilum* orchid (Ferry, 2011). Umbel inflorescence is where the flowers are cluster with pedicels that arise from same point of peduncle. They have a similar length of

pedicel and a very competitive growth between the flowers. Usually the outside flower wills flowering first and innermost flower flowering later. Very few of orchid species belong to this group. *Neobenthamia gracilis* is one of the examples of umbel inflorescence. Raceme inflorescence is where a flower is attached to pedicel along the rachis. In raceme inflorescence, the flowering will start form innermost to outermost flower. This can be seen in *Brassia caudata* orchid. In certain species, the pedicel which is a stalk is absence and the flowers are fixed on rachis. The flower that fixed on rachis is spike inflorescence. The flower will open from the bottom to the top of rachis. *Tipularia discolour* which is terrestrial orchid is belong to this group. The panicle inflorescence is comprised of branches of many flowers. The flowering will start from outermost flower to innermost flower. *Oncidium* is an example of orchid that has panicle inflorescence (Ferry, 2011).

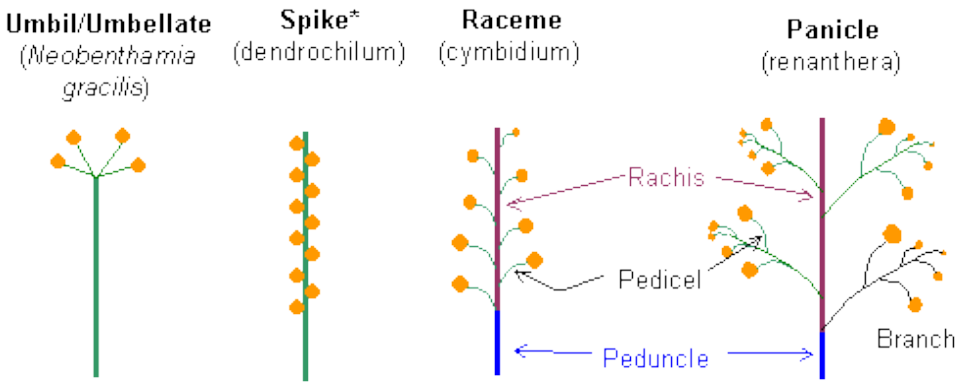

Figure 2.4: Several forms of inflorescence in orchids. Retrieved from http://www.canberraorchids.org/tips.html.

2.1.4 Pod Harvesting

One orchid seed pod contains millions of seeds. Orchid seeds are very tiny and dust-like (Thomas, 1992). It takes a long time (4 – 10 months) for orchid pod to be mature after pollination (Norberto, 2010). Time for the orchid seeds and pods to be matured are depends on genus and species of orchids. An optimum harvesting time for *Phalaenopsis*

pod is between 110 to 120 days after pollination takes place. At 111 days, the green pod are recommended to be harvested. An optimum harvesting time for *Vanda* pod is between 150 to 195 days and is recommended to harvest at 182 days after pollination (Kay, 2000). Orchid pod shows the signs of maturity when its green colour turn yellowing or there is a presence of crack (Norberto, 2010).

2.2 *Phalaenopsis* sp.

Phalaenopsis is one of the orchid genus that known as Moth Orchids. Moth orchids are very popular among the gardeners or commercial growers. *Phalaenopsis* species is native to Borneo. There are two groups of *Phalaenopsis* orchids which are long stems and short stems. *Phalaenopsis* with long stems has inflorescence branches and round shape of flowers while the *Phalaenopsis* with short stems do not have characteristic of inflorescence branches and waxy shape of flowers. Most of *Phalaenopsis* species are epiphytes and a few are lithophytes. *Phalaenopsis* shows the monopodial growth (Alan, 2003).

2.2.1 *Phalaenopsis bellina*

Figure 2.5*: Phalaenopsis bellina*. Retrieved from http://www.ranwild.org/Phalaenopsis/module/species/bellina/Especieshead.html.

In this project, *Phalaenopsis bellina* is used as this *phalaenopsis* species is easily found in Borneo especially in Kuching area. *Phalaenopsis bellina* is known as Normah orchid as this orchid is a state flower of Sarawak. According to Official Website of The Commission of The City of Kuching North (2014), *Phalaenopsis bellina* was declared as state flower of Sarawak on 28th August in 1983. Many local people own this species in their garden. However, it is not easy to plant this species as it is a very sensitive plant. *Phalaenopsis bellina* requires many preferred condition for them to grow. Little research has been done by researcher for this species. The size of the flower is between 5 cm to 6 cm. Its flower has fragrance and the flowering can last for 3 months (Kader and Delseny, 2008). It has large round leaves and waxy. These characteristics make it unsuitable for planting in pots.

2.3 *Vanda* sp.

Vandaceous has three types of genera which are *Vanda, Ascocentrum,* and *Ascocenda*. *Ascocenda* is a hybrid between *Vanda* and *Ascocentrum* (Robert, n.d). *Vanda* orchid shows the monopodial growth habit like *Phalaenopsis* orchid. *Vanda* can be found in Thailand, Borneo and Philippines. *Vanda* orchid are among orchids that have high prize in horticulture. This is because this species has variety colours and large flowers. The flowers of *Vanda* bloom six or more times in a year (Pavallekoodi *et al*., 2012). *Vanda* can be divided into three group based on their leaves shape which are strap leaved plant, terete plant, and semi-terete plant. The strap leaved *Vanda* has flat and leathery leaves. *Vanda coerulea* and *Vanda dearei* are among including in this group. Terete *Vanda* has a tapered and pencil-shaped leaves which are circular in cross section. *Vanda teres* is most common species that show this character. Semi-terete *Vanda* has a tapered and pencil-shaped leaves but not completely circular in cross section (Robert, n.d).

2.3.1 *Vanda dearei*

Vanda dearei in Borneo can be found in Kinabatangan and Tenom in Sabah, Kuching in Sarawak, and Sekayan River and Kutai in Kalimantan. *Vanda dearei* has pale yellow flowers. *Vanda dearei* is known for its source of yellow colour in *Vanda* hybrids. *Vanda dearei* shows the epiphyte growth habit. This species has flowers which are long lasting for about one to two weeks and very fragrant (Jualang *et al*., 2014).

Figure 2.6: *Vanda dearei*. Retrieved from http://mics-wildorchid.blogspot.com/.

2.4 Micropropagation of *Phalaenopsis* sp.

The micropropagation of *Phalaenopsis* from immature seeds by green pod culture is a fast process. In these techniques, the immature seeds in a green pod are collected from plant after fertilization (Ramnath *et al.,* 2005). Green pod culture techniques used the green capsules (immature seed) that are fresh and the seeds within it are naturally sterile. So, the seeds do not need sterilization. Only the surface of outside green capsule needs to be sterilized. The seeds from the green capsules can germinate at the faster rate (Sheena, 2000). If the seeds sown are not mature enough, the germination process will be slower or seed will not germinate at all.

2.4.1 Seed Germination

2.4.1.1 *In Vivo* Seed Germination

In vivo, plant is produced sexually through seeds or vegetative. Orchids grows in natural habitat require the presence of symbiotic fungi for the seeds to germinate. This fungi supply sugar to orchid seed for germination until the seedling has enough chlorophyll to make its own sugar and able to sustain itself (Thomas, 1992). In nature, *Phalaenopsis* take a longer time to grow as orchid seed are hard to germinate.

2.4.1.2 *In Vitro* Seed Germination

In vitro, plant is produced asexually. Knudson (1922) has proved that orchid seeds are able to germinate in laboratory without the presence of fungus. Knudson (1922) showed that fungus was converting the starch in medium into sugar and this sugar was used for germination. Knudson replaced starch with sugar and orchids grow very well without the fungus (Thomas, 1992). Through *in vitro*, native orchids like *Phalaenopsis* can be grown easily.

2.5 Microprogation of *Vanda* sp.

2.5.1 Protocorm

There are many factors influenced the development of protocorm. The 4 factors include source of explants, the condition during culturing, type of basal media used, and types of organic additives used. Young protocorms are preferred to be chosen as a source explants. Young protocorms differentiate actively and has high metabolic activity. The study of *Vanda dearei* by Jualang *et al*. (2014) has shown that the development and growth of protocorms favoured low amount of concentration and low complexity of nutrient. The

presence of organic additives also helps to enhance the growth and development of protocorms. Study by Islam *et al*. (2011) has reported that the protocorm production from germination of seeds was enhanced by supplemented the media with potato extract.

2.6 Types of Basal Media

Types of basal media used influenced the development of seed germination and the seedling growth. There are three types of basal media used in this study which are Murashige and Skoog (MS) media, half Murashige and Skoog (1/2 MS) media, and Gamborg B5 media (B5). All these media consists of macroelements, microelements, and vitamins.

Macroelements composed of carbon, oxygen, hydrogen, nitrogen, potassium, phosphorus, calcium, magnesium, and sulphur. Macroelements are required in large amount which is more than 0.5 mM/L for plant growth and development (Abobkar and Ahmed, 2012).

Microelements are needed in trace amounts which are less than 0.05 mM/L for plant growth, development and diverse roles. Microelements composed of manganese, iodine, copper, cobalt, boron, molybdenum, iron, zinc, nickel, and aluminium.

Vitamins are added to improve the plant growth in culture but not really necessary. Thiamin which is known as vitamin B1 is essential for many plant species in culture. Thiamin is needed for metabolism of carbohydrate and biosynthesis of some amino acids. Nicotinin acid (niacin), pyridoxine (B6), glysine and myo-inositol are examples of vitamins used in media culture (Abobkar and Ahmed, 2012). Myo-inositol is a sugar alcohol and important for cell wall development. It is usually added to media for monocots plant like orchids.

2.7 Organic Additives

The addition of complex organic substances helps to promote the seed germination and seedling growth. There are four types of organic additives used in this study which are coconut water, tomato homogenate, banana pulp, and potatoes homogenate.

In micropropagation techniques, coconut water is added into the media to increase the cell proliferation. Coconut water contains high amount of potassium and antioxidants. It also contains cytokinins that help to promote plant cell division and growth. Usually, the coconut water is taken from unripe coconut (Joseph, 2008). Joseph (2008) has stated that Mariat (1951) was the first person who published on the use of coconut water in orchid seed germination. Mariat has added coconut water at concentration of 2% (v/v) in a media and this has caused the seedlings turn yellowish green. However, it did not inhibit the seed germination and development.

Tomato homogenate media is effective for increasing the rate of seed germination and enhanced the growth of protocorms. Tomato contains vitamin, vitamin k, mineral, and lycopene that can affect the development of cells. Based on previous study, high amount of tomato homogenate in the cultured media can produce higher number of seed germination until they show the stages of regeneration. Low concentration of tomato homogenate in the media was found to inhibit the early stage of seed germination in orchid. Addition of tomato homogenate at concentration of 5% in half strength of Murashige and Skoog (MS) media for *Geodorum densiflorum* (Lam) Schltr orchid produced 100% of seed germination and formed dark green protocorm (Muthukrishnan *et al.*, 2013).

Banana pulp also helps in promoting the growth of orchid seedling. Banana in its powder state was first used in a medium for orchid seed germination in Brazil. The media that containing banana are easy to recognize because of their darker colour. Pulp from a ripe