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JUDUL: PRODUCTION OF HYBRID ORCHID: IN VITRO SEED GERMINATION OF Renanthera bella x Vanda helvola; Paraphalaenopsis denevei x Phalaenopsis bellina AND P. dearei x V. helvola.

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PRODUCTION OF HYBRID ORCHID: *IN VITRO* SEED GERMINATION OF *Renanthera bella* x *Vanda helvola; Paraphalaenopsis denevei* x *Phalaenopsis bellina;* AND *Vanda dearei* x *Vanda helvola*

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DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

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I was inspired to take up this project as it is something to do with commercialization of a nature product and orchid is the prime resource of Sabah but little has been done into hybrid production. It took me a hundred mile a way to Tenom with patient and courage, just to get this project done. But as time pass by, I learnt to appreciate the opportunity it takes to excel in this arena.

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ABSTRACT

A hybrid is a result of the union, whether naturally occurring or manmade, of two plants of different genetic backgrounds. Assisted cross-pollination was carried out by placing the pollen of one orchid plant onto the stigma of another orchid plant, resulting in the formation of seeds which grow into a new hybrid. The *in vitro* seed germination of hybrid seeds of *Renanthera bella* x *Vanda helvola; Paraphalaenopsis denevei* x *Phalaenopsis bellina;* and *V. dearei* x *V. helvola* were done by using half strength MS medium supplemented with 10% v/v of coconut water, 10% w/v of tomato juice, 10% w/v of potato homogenate and a control treatment. For 30% germination, *R. bella* hybrid seeds took 10 days after culture on half-MS supplemented with coconut water, *P. denevei* hybrid 14 days after culture on half-MS without additive, and *V. dearei* hybrid took 14 days after culture on half-MS supplemented with potato homogenate. All cultures from the entire studies were maintain under 24 hour light conditions at 24±2 ^{0}C. The analysis of variance (ANOVA) confirmed that there is a significant effect of complex additives on the *in vitro* germination of all the three hybrids seeds at 20-60 days after culture.

PENGHASILAN ORKID KACUKAN: PERCAMBAHAN BENIH SECARA *IN VITRO* *Renanthera bella* x *Vanda helvola; Paraphalaenopsis denevei* x *Phalaenopsis bellina;* DAN *V. dearei* x *V. helvola*

ABSTRAK

Kacukan adalah terhasil daripada penyatuan dua induk tanaman yang berbeza dari segi latarbelakang genetiknya samaada secara semulajadi atau dengan bantuan manusia. Pengacukan secara berbantu dilakukan dengan memindahkan debunga jantan daripada satu jenis orkid kepada debunga betina (stigma) suatu orkid yang lain menyebabkan terhasilnya benih yang akan membesar menjadi orkid kacukan yang baru. Percambahan biji-benih secara *in vitro* antara *Renanthera bella* x *Vanda helvola; Paraphalaenopsis denevei* x *Phalaenopsis bellina dan V. dearei* x *V. helvola* telah berhasil dicapai dengan menggunakan medium setengah-MS yang dicampurkan dengan bahan kompleks seperti air kelapa muda dengan kepekatan 10% v/v, jus tomato 10% w/v, homogenat kentang 10% w/v dan satu rawatan kawalan. Kacukan *R. bella* bercambah pada kadar 30% dalam tempoh 10 hari selepas disemai (media separuh-MS campur 10% air kelapa), sementara *P. denevei* 14 hari selepas disemai (media separuh-MS tanpa bahan tambahan) dan kacukan *V. dearei* pula bercambah selepas 14 hari disemai (media separuh-MS campur 10% homogenate kentang). Semua piring petri disimpan dalam bilik percambahan dengan bekalan cahaya selama 24 jam dan suhu bilik 24±2 ^{0}C. Analisis ANOVA membuktikan bahawa terdapat perbezaan bererti kesan penambahan bahan kompleks kepada medium percambahan *in vitro* terhadap ketiga-tiga orkid hybrid pada 20-60 hari selepas pengkulturan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

α	Significant level
"	Inch
^{0}C	Degree Celsius
AC	Activated Carbon
ANOVA	Analysis of variance
cm	Centimeter
CRD	Completely Randomized Design
CW	Coconut Water
HCl	Hydrocloric acid
K	Potassium
km	Kilometer
mm	Milimeter
MS	Murashige and Skoog, 1962
N	Nitrogen
NaOH	Sodium hydroxide
P	Phosphate
PH	Potato homogenate
TJ	Tomato juice
v/v	Volume per Volume
w/v	Weight per Volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

The development of orchid hybrids in Malaysia is closely related to the activities of the Malayan Orchid Society, which was grown to become the Orchid Society of South East Asia (OSSEA). Based on statistical studies by MARDI (2001), new hybrids increased at the rate of at least 1,000 per year. A hybrid is a result of the union, whether naturally occurring or manmade of two plants of different genetic backgrounds (Sheehan, 2001). They occur principally on the species and generic levels. A primary hybrid is produced when two species are brought together. Making of hybrid in orchid hybridization, cross-pollination is carried out by placing the pollen of one orchid plant onto the stigma of another orchid plant, resulting in the formation of seeds which grow into a new hybrid. The ease with which free gene flow is permitted across the taxanomic limits and hybrid embryos rescued have added to new dimensions in orchid breeding according to Vij (1998) and Kishor *et al.* (2005).

The discoveries of aseptic seed germination by Luis Knudson in the 1920s, polyploids dimensions and their role in breeding in the 1950s, and meristem propogation in the 1960s have all had a significant and stimulatory effect on orchids and orchid production (Sheehan, 2001). These discoveries have enables the grower to produce hybrids faster and in greater numbers than ever before. The overwhelming popularity of hybrid orchids is due to variety of reasons such as superior quality, easy cultivation, free-blooming habit, better shapes, colours and longer shelf-life (Teo, 2002; Soon, 2005). Free-blooming or freedom of flowering in orchid plants appears to be affected by polyploidy, the flowering characteristics of the parents and their behavior as seedlings (Soon, 1980). According to Soon (1980) the common effect of polyploidy is to increase the vegetative portion of the plants, making them lushier and more vigorous than the

corresponding diploids when they are fully grown.

According to Nash (2003) and Johnson and Kane (2007), an estimate of 90% of all the orchids sold in America prior to year 2003 were *Phalaenopsis* species and hybrids. However, the demand for other orchid genera has increased as consumer become accustomed to growing and displaying orchids at their homes. Due to that reason breeders are competing to produce new generation of hybrid orchid.

1.2 Justification

The production of hybrid orchid by using the local and endemic species to Borneo is timely as there are very few studies on this subject in Sabah. As we know that Sabah is very unique with its mega-biodiversity and popular for its conservation work. Nevertheless, our effort should be diversify to produce more product through the advancement of the current technologies and trends. *Renanthera bella*, for example is the endemic species in Sabah but has been exploited in other countries. Conservation work can be maintained to sustain the germplasm collections but breeding of new hybrid should be carried out to meet the current demand as well as to boost our economy.

1.3 Objective

The objective of this study was to obtain the right medium for efficient germination of the three orchid hybrid seeds of *Paraphalaenopsis denevei* x *Phalaenopsis bellina*, *Renanthera bella* x *Vanda helvola* and *V. dearei* x *V. helvola.*

CHAPTER 2

LITERATURE REVIEW

2.1 Orchid Distribution

The Orchidaceae is among the largest families in flowering plants. Species counts ranged from 17,500 – 35,000 (Hagsater and Dumont, 1996) but well-documented report shows only about 20,000 species (Atwood, 1986; Dressler, 1993; as cited by Hagsater, 1996). According to Dressler (1993; as cited by Hagsater 1996), orchids are far more diverse in the tropics than in any other ecosystem which recognized 803 genera with a total of 19,501 species for the entire family. Orchids have a very wide range of distribution. They are found to occur in all parts of the world except, perhaps, in the Antarctica. Orchidaceae is also the most successful plant families among all the families of flowering plants after Compositae.

2.2 Orchid of Borneo

Borneo is the third largest island in the world. The size of this island is over 1,300 km long and 950 km wide with the area of nearly 740,000 km square. Politically Borneo shared by three countries, Malaysia, Indonesia and Brunei Darussalam. Borneo is known to be a center of diversity of many floral species and also as the "Island of Fruits", but could equally referred to as the "Orchid Island". The largest family in the region is Orchidaceae with 3,000-4,000 species, comprising 12-16% of the flora. Lamb (1991) has estimated that 2500-3000 orchid species are found in Borneo, equivalent to 10% of the world's orchids or 10-12% of the Malesian flora and 75% of the Malesian orchid flora. It was estimated that about 30-40% are thought to be endemic to the island (Chan *et al.*, 1994). Wood *et al.* (1993) listed over 1400 species in 147 genera

and documented nearly 700 species in 121 genera of orchids are dominant to Mount Kinabalu areas. This represents nearly a fifth of all the vascular plants, and perhaps nearly a half of the Bornean orchids on this mountain alone. Epiphytic orchids are most common in the emergent layer of the rainforest, often in the forks and along the main branches of trees. Species found to be the *Phalaenopsis*, *Paraphalaenopsis*, *Vanda*, *Renanthera*, *Dendrobium*, *Grammatophyllum*, *Dimorphorchis*, *Bulbophyllum* among others. Besides rainforest, the orchid also can be found in other habitat such as riverine and riparian forests, mangrove forests, peatswamp forest, limestone areas, heat forests and lower montane rainforest.

2.2.1 *Paraphalaenopsis denevei*

Paraphalaenopsis denevei (Figure 2.1) is an epiphytic monopodial orchid and is endemic to Borneo especially Kalimantan (Chan *et al.*, 1994). This species is morphologically similar to *Phalaenopsis* and was a long time considered as species of that genus. The generic name is derived from the greek para and "phalaenopsis" referring to the close affinity of the two genera. This orchid can be found in the west Borneo (Figure 2.2), on the riverside trees in lowland primary forests at elevation around 300 m altitude. Both *Phalaenopsis* and *Paraphalaenopsis* flowers are similar, but the leaves of *Paraphalaenopsis* are cylindrical and long (from 35 cm up to 3 m in cultivation) with a short stem carrying three to six fleshy, elongate, cylindrical, canaliculated leaves (Figure 2.3). It has shorter inflorescence that is crowded with up to 13 sweetly scented flowers with approximate diameter of 4 cm across. In cultivation, this species flowers three to four times a year.

P. denevei can be use as a female parent in hybrid production for potted plant because of its outstanding rat-tailed (terete) leaf. According to Soon (2005), the *Paraphalaenopsis* is interfertile with *Ascosentrum*, *Aerides*, *Vanda*, *Renanthera* and *Rhysncostylis* besides *Phalaenopsis*. The first *P. denevei* hybrid was registered in 1950, a cross with *Arachnis* Maggie Oei. Another positive contribution of *P. denevei* to hybridization are fine coloration, heavy substance, circular arrangement of it flowers and durability (Soon, 2005).

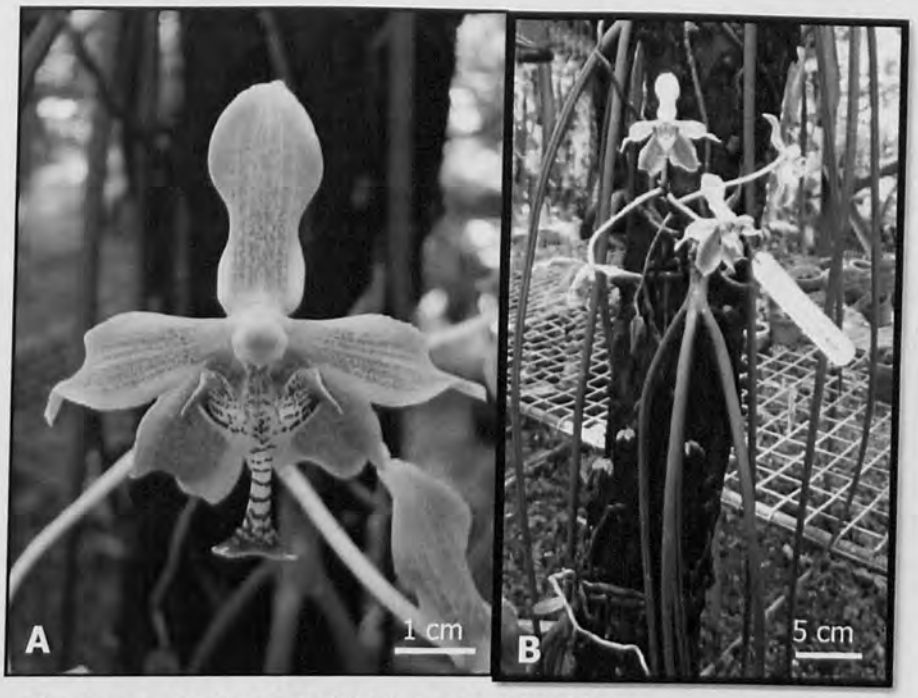

Figure 2.1 *P. denevei*: (A) Flower and (B) Plant

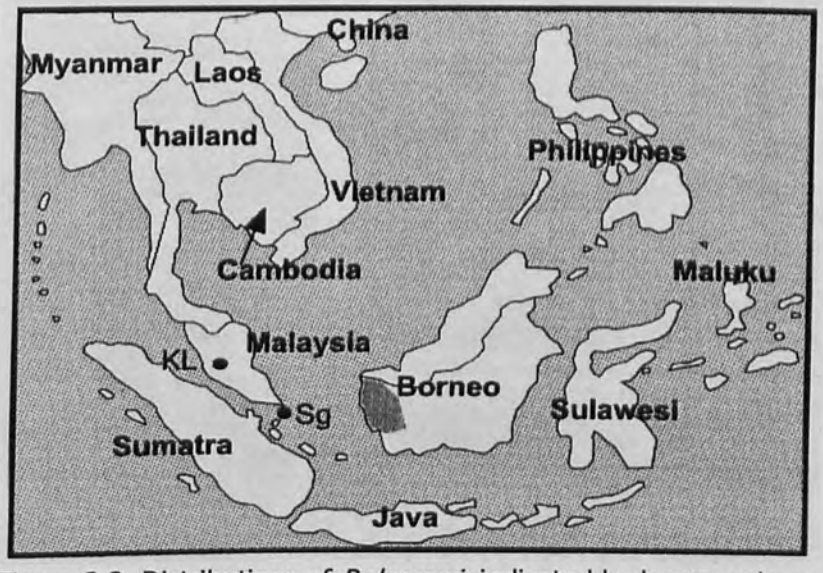

Figure 2.2 Distributions of *P.denevei*, indicated by brown colour
Source: Taken from O'byrne, 2001

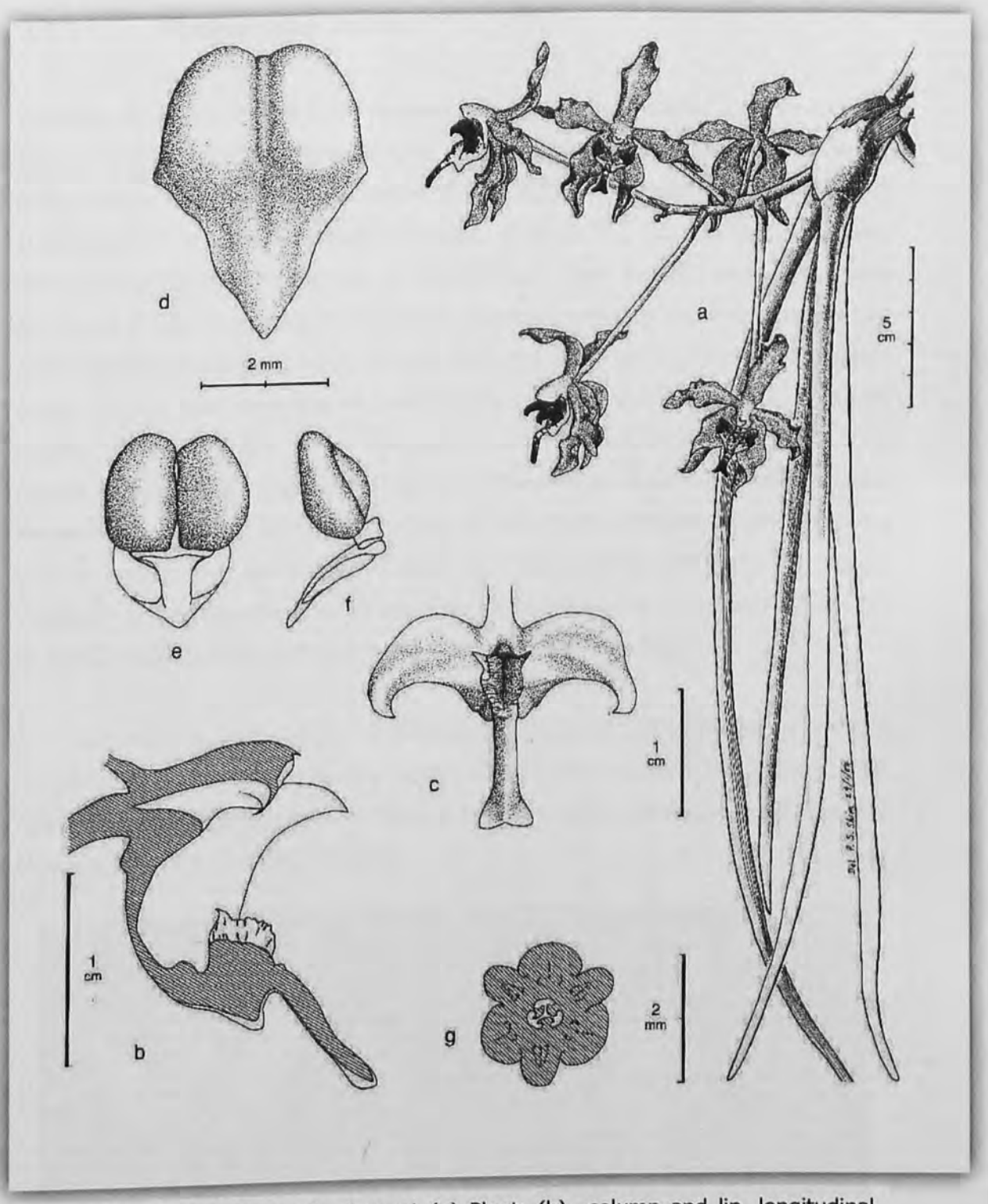

Figure 2.3 *Paraphalaenopsis denevei*. (a) Plant; (b) column and lip, longitudinal section; (c) lip spread out; (d) anther, front view; (e) pollinarium, front view; (f) pollinarium, lateral view; (g) ovary, transverse section.

Source: Taken from Chan *et al.*, 1994

2.2.2 *Phalaenopsis bellina*

Phalaenopsis bellina (Figure 2.4), commonly known as 'Lundu Orchid' or Norma Orchid (in honoured of the wife of Sarawak Chief Minister), is endemic to the island of Borneo and grows in Sarawak and Kalamantan (Figure 2.5). This flower was picked up the state flower of Sarawak. According to Beaman *et al.* (2001), this species was formally describe as a variety of *P. violacea* by Reichenbach before it gets it real name. Plants are found in nature growing in a pendulous fashion, with 5-8 cm green flowers that have purple markings on the lower sepals. They are extremely fragrant and can bloom trough out the year. According to Kaiser (1993; as cited by Beaman *et al.*, 2001) the lemony fragrance of this species composed almost entirely 64% geraniol and 32% linalool. *P. bellina* is a monopodial growth type of orchid, an erect to ascending growing rhizome produces from the top one or two alternate, thick and fleshy, elliptical leaves a year. In nature, they are typically fond of warm temperatures (20 to 35 °C), but are adaptable to conditions more comfortable for human habitation in temperate zones (15 to 30 °C). *P. bellina* requires high humidity (50-70%) and low light.

According to Soon (2005), *Phalaenopsis* is the most hybridized genus amongst the monopodial orchids with several thousand interspesific hybrids. Hybridization of *P. bellina* may utilize its advantage of being sturdy potted plant, early flowering, non-stop blooming as well as it strong fragrance.

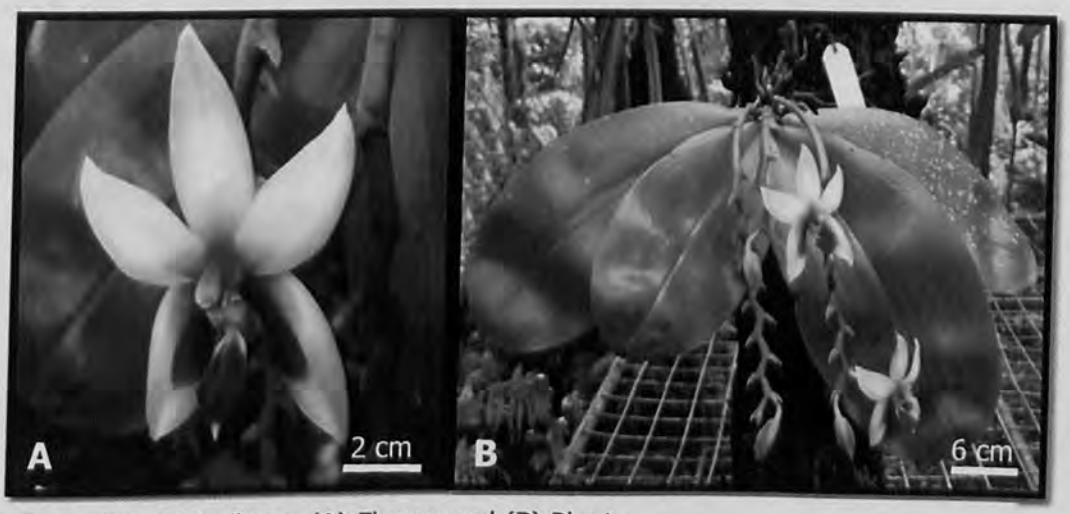

Figure 2.4 *P. bellina* : (A) Flower and (B) Plant

Figure 2.5 Distributions of *P.bellina; indicated by* brown colour
Source: Taken from O'byrne, 2001

2.2.3 *Renanthera bella*

Renanthera bella (Figure 2.6) is an epiphytic orchid which bears beautiful flowers; alluded to by the specific name of bella. The generic name is derived from the Latin *renes*, means kidney and the Greek *anthera,* means anther describing the kidney-shaped pollinia of this genus. The specific name *bella* derived from the Latin *bellus*, which means beautiful, referring to the attractive flowers. This orchid is endemic to Sabah and is listed under Appendix I of the Convention on International Trade in Endangered Species of wild flora and fauna (CITES) for which no trade is allowed except for nursery-raised flask seedlings (Chan *et al.*, 1994).

Figure 2.6 *R. bella*: (A) Flower and (B) Plant

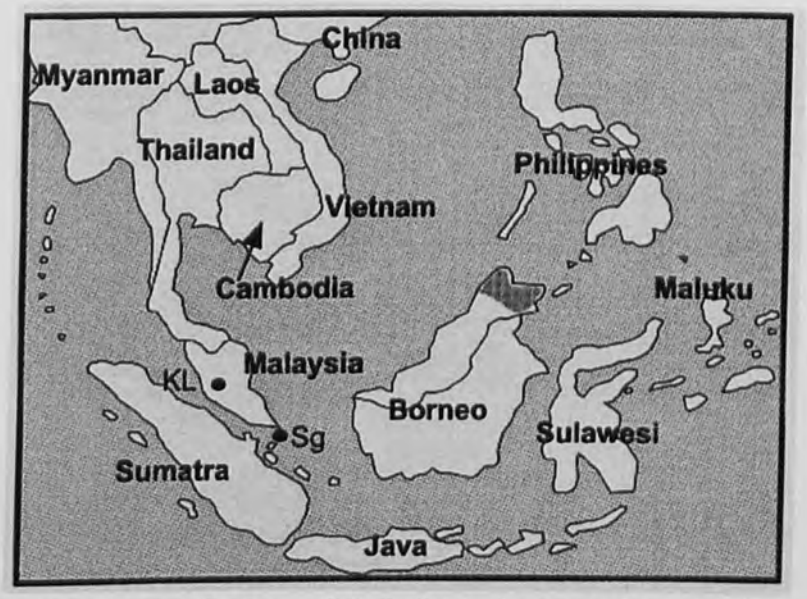

Figure 2.7 Distributions of *R. bella; indicated by* Brown colour.
Source: Taken from O'byrne, 2001

This rare species is confined to a few location in the hill-country of Sabah (Figure 2.7), growing from 800-1100 m altitude (O'byrne, 2001) and is known only from Mount Kinabalu (Chan *et al.*, 1994). It prefers to grow in slightly shaded places, often low down near the ground. Aerial roots hang down from the platform upon which the plant is situated, the orchid's stem is covered with dead greyish-brown leaves below and is leafy above (Figure 2.8). The flower stalk, or inflorescence, is horizontal or drooping

and may carry up to 25 delicate red flowers. The sepals and petals of the flower are narrow, yellowish-cream below but with many pink or crimson blotches above. The lip petal is fleshy with three lobes and it is also marked with dark red blotches. Flowering is in February-March and July-September (Chan *et al.*, 1994). This species is considered highly endangered species due to over-collecting (O'byrne, 2001).

According to Soon (2005), *Renanthera* has been bred with almost every single genus of *Vanda-Arachnis* tribe although some combinations are difficult to achieve (such as *Phalaenopsis* and *Doritis*). *R. bella* has shown to have dominant red colour, floriferousness, well-displayed inflourescence with many side branches, vigour and in most instances, the ability to withstand full sun (Soon, 2005). Its longer flower stalk has been an advantage to produce a better breed for cut flower industry.

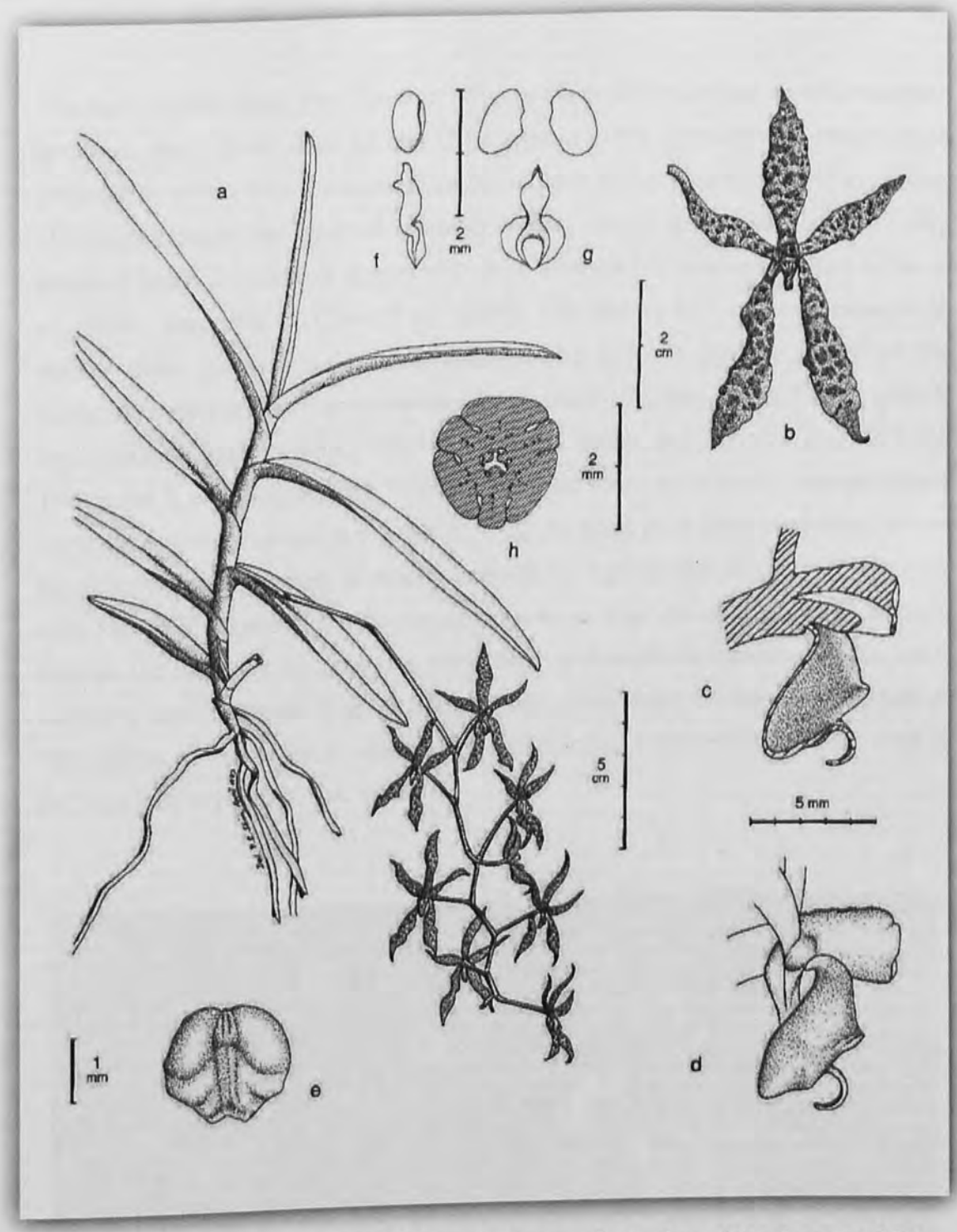

Figure 2.8 *Renanthera bella* (a) Plant: (b) flower, front view; (c) column and lip, longitudinal section; (d) column and lip, lateral view; (e) anther; (f) pollinarium, lateral view; (g) pollinarium, front view; (h) ovary, transverse section.

Source: Taken from Chan *et. al.*, 1994

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