THE REPRODUCTIVE BIOLOGY OF *Vatica yeechongii* Saw (DIPTEROCARPACEAE)

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2017

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DISSERTATION SUBMITTED IN FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2017

UNIVERSITY OF MALAYA
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THE REPRODUCTIVE BIOLOGY OF *Vatica yeechongii* Saw

(DIPTEROCARPACEAE)

ABSTRACT

Vatica yeechongii is a critically endangered and endemic species in Peninsular Malaysia (Chua *et al.*, 2010). Land use change is a threat to this species as the population only exist in Sungai Lalang Forest Reserve, Selangor and Setul Forest Reserve, Negeri Sembilan. To date, there are less than 50 trees with diameter at breast height (DBH) more than 2 cm recorded from both areas. The aim of this research is to study the flowering and fruiting pattern, breeding system and potential pollinators for this species. *Vatica yeechongii* flowers annually but there are few trees producing flowers twice a year. *Vatica yeechongii* begins to produce flowers between February and March, while fruits mature and fall between July and August. The total duration period from trees started to develop flowers until mature fruits fall took about 21-22 weeks. Flower started to open at early dawn around 0530 hour and lasted for four to five hours. *Vatica yeechongii* is protandrous, where the anthers started to dehisce pollens upon floral opening and the stigmas become receptive 30 minutes later. Dehiscence of petals and anthers began as early as 0930 hours. *V. yeechongii* is an outcrosser and self-incompatible species. Insects from the family Apidae (*Trigona* sp.) were seen to be more effective as pollinators due to their hairy abdomens and legs which were believed to be able to carry more loads of pollens. The pollen grains are tricolpate, with thick and coarsely reticulate exine, viable for almost two days.

Keywords: Vatica, floral phenology, breeding system, pollination biology, Apidae

BIOLOGI PEMBIAKAN *Vatica yeechongii* Saw (DIPTEROCARPACEAE)

ABSTRAK

Vatica yeechongii adalah spesies sangat terancam dan endemik di Semenanjung Malaysia (Chua *et al.*, 2010). Perubahan status guna tanah merupakan ancaman kepada spesies ini memandangkan populasi ini hanya wujud di Hutan Simpan Setul dan Hutan Simpan Sungai Lalang. Setakat ini, terdapat kurang daripada 50 pokok yang mempunyai diameter pada paras dada lebih daripada 2 cm direkodkan daripada kedua-dua kawasan tersebut. Tujuan kajian ini dijalankan adalah untuk mengkaji corak pembungaan dan pembuahan, sistem pembiakan serta agen pendebungaan spesies *Vatica yeechongii*. *Vatica yeechongii* berbunga setiap tahun, namun terdapat sebilangan kecil pokok mampu menghasilkan bunga dua kali setahun. Spesies ini mula menghasilkan bunga di antara bulan Februari dan Mac, manakala buah matang dan gugur di antara bulan Julai dan Ogos. Jangkamasa tempoh daripada pokok mula mengeluarkan bunga sehingga buah matang gugur adalah kira-kira 21-22 minggu. Bunga *Vatica yeechongii* akan mula berkembang seawal jam 0530 pagi dan kekal kembang untuk tempoh empat ke lima jam sahaja. Spesies ini adalah protandri, di mana anter membebaskan debunga apabila bunga mula berkembang, dan stigma reseptif selepas 30 minit. Kelopak bunga dan anter akan mula gugur seawal jam 0930. *Vatica yeechongii* adalah spesies pembiakbakaan luar dan ketidakserasian sendiri. Serangga daripada family Apidae (*Trigona* sp.) berkemungkinan besar adalah agen pendebungaan yang berkesan berdasarkan kepada abdomen dan kaki yang berbulu dipercayai membolehkannya membawa banyak debunga. Debunga adalah trikolpat, eksin tebal dan retikulat kasar serta kemandirian kekal hampir 2 hari.

Kata kunci: Vatica, fenologi bunga, sistem pembiakan, biologi pendebungaan, Apidae

ACKNOWLEDGEMENTS

First, I would like to express my thanks and sincere appreciation to my supervisor Associate Professor Dr. Noorma Wati Haron and my advisor Dr. Richard Chung Cheng Kong for their invaluable advice, patience, encouragement and enthusiasm towards completing this study; Dr. Lillian Chua Swee Lian (the Director of Forest Biodiversity Division, FRIM) for her critical comments, support and assistance throughout the project.

I would like to express my gratitude and appreciation to all the staffs of Forest Health and Conservation Programme, FRIM for their assistance during fieldwork especially to Amir, Hamidi, Zamli, Azmi, Mohd Zian, Ayau, Alang, Zarul and Rafizzi.

I would like to extend my thanks to the Malaysian Meteorological Service Department; State Forestry Department of Selangor and Negeri Sembilan; Phon Chooi Khim (FRIM) and Nur Zati Akma (FRIM) for the identification of insect specimens; Dr. Ng Yong Foo (UKM) for the thrips identification; Roslee Halip (ISB, UM) and Abdul Azizi Jalil (FRIM) for the scanning electron microscope (SEM) work; Aidil Nordin (FRIM) who prepared the illustration and staff of Genebank and Seeds Centre at MARDI for lab assistance and permission to use their facilities.

I greatly acknowledge Forest Research Institute Malaysia (FRIM) for sponsoring my study and Malaysian Government Research and Development Grant MPKSN/IRPA for research grant under the project entitled: Conservation Monitoring of Rare and Threatened Plants of Peninsular Malaysia.

Finally, I would like to express my deepest gratitude to my family members and my dear husband Zulkefli Hassan for the great support physically and morally given in pursuing this Master's thesis. Not forgetting to my beloved children, Awfaa Addeena and Aqeef Aiman, thank you for understanding and mama dedicate all this work to both of you.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviation	Word
^{o}C	degree Celcius
=	equal to
>	greater than
<	less than
%	percentage
a.s.l.	above sea level
et al.	and others
b.a.	boric acid
cm	centimeter
DBH	diameter at breast height
FR	Forest Reserve
LM	light microscope
µm	micrometer
mm	millimeter
N	number
r.p.m	rotation per minute
SEM	Scanning electron microscope
sp.	species
SD	standard deviation

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

Dipterocarpaceae is a family of 17 genera and approximately 500 species (Ashton, 1982) distributed mainly in tropical lowland rainforest trees. Its distribution is pantropical, from South America to Africa, the Seychelles, Sri Lanka, India, Indochina and Southeast Asia. The greatest diversity and abundance of this family is in Borneo. Members of the family are found in wide-ranging habitats from the coasts to hills of the tropics at altitudes below 1800 m. Dipterocarpaceae is a major ecologically component of the lowland rain forests. Members of this family dominate the forests, constitute up to 10% of all tree species and 80% of all emergent individuals (Ashton, 1982). A few occur in the lower montane rain forests.

Dipterocarps also occur in seasonal tropical forests and savannas in India, Sri Lanka, Madagascar, Africa and the Guayana Highlands but often lack the diversity, and this family dominates the aseasonal forests of Southeast Asia (Appanah, 1993). Within the Indo-Malayan region, Dipterocarpaceae has its centre of diversity in a region called Malesia encompassing Peninsular Thailand, Malaysia, Singapore, Indonesia, Philippines and the island of New Guinea. Malesia has about 10 genera and 387 species. In Peninsular Malaysia, of the 165 taxa comprising 155 species, 34 taxa are endemic. Ninety taxa are common to Peninsular Malaysia, Sabah and Sarawak while the remaining 41 taxa extend their distributions to Sumatra and Philippines. The genus *Vatica* is one of the larger natural groups of dipterocarps, comprising 65 species (Ashton, 1982). This genus occurs in Sri Lanka, southern and eastern India, Myanmar, Thailand, Indochina, south China and Malesia.

Of the 164 taxa, ninety two taxa of dipterocarps have threatened category nationwide (Critically Endangered, Endangered and Vulnerable); of these twenty two are

endemic to Peninsular Malaysia. Of the 92 threatened taxa, 14 species are from the genus *Vatica*, namely *Vatica flavida*, *V. havilandii*, *V. hullettii*, *V. lobata*, *V. maingayi*, *V. mangachapoi* ssp. *mangachapoi, V. odorata* ssp. *odorata, V. pallida*, *V. perakensis*, *V. ridleyana*, *V. scortechinii*, *V. stapfiana, V. venulosa* and *V. yeechongii*,. Six species, *Vatica flavida*, *V. hullettii, V. lobata, V. pallida*, *V. scortechinii* and *V. yeechongii* are endemic to Peninsular Malaysia.

The phenomenon known as mast flowering or general flowering occur when the majority of dipterocarp species exhibit simultaneous heavy mass flowering, followed by mast fruiting over a wide region. This phenomenon can occur at intervals of 2 to 10 years and may last about six months. General flowering or mast flowering of dipterocarps followed by mast fruiting presents a unique phenomenon in lowland dipterocarps forests in Southeast Asia (Sakai, 2002).

Mast flowering was originally believed to be confined to dipterocarps. According to Sakai *et al.* (1999), this phenomenon takes place at the community level and is not restricted to dipterocarps. Other plant families also are heavily flowered during a general flowering of dipterocarps. Wood (1956) recorded heavy flowering of non-dipterocarp trees at the same time with dipterocarps in 1955. Other families that synchrony flowering with Dipterocarpaceae are Euphorbiaceae, Annonaceae, Leguminosae, Burseraceae and Myristicaceae (Appanah, 1985; Momose *et al*., 1998; Sakai *et al.,* 1999). The flowering intensity of dipterocarps also differs. The flowering for the emergent and main canopy species can be heavy over the whole crown, but for the understorey species like *Hopea* and *Vatica*, flowering was more often restricted to a few branches (Wood, 1956).

In Peninsular Malaysia, during the mast flowering and fruiting of dipterocarps in the middle of year 2002, a specimen of *Vatica* was collected from the Sungai Lalang Forest Reserve (FR), Selangor. This has been identified as a new species under the section *Vatica*.

The new species is described as *V. yeechongii* Saw, named after Mr. Chan Yee Chong, the late para-taxonomist at the Forest Research Institute Malaysia.

1.2 Problem statement

According to the Malaysia Plant Red List (Chua *et al.*, 2010), *Vatica yeechongii* is categorized under Critically Endangered (CR A4c, D2). *V. yeechongii* is rare and endemic to Peninsular Malaysia as it only occurs in two localities in this world; Setul Forest Reserve (FR) in Negeri Sembilan and Sungai Lalang Forest Reserve (FR) in Selangor (Figure 1.1). To date, there are less than 50 trees with diameter at breast height (DBH) more than 2 cm recorded from both areas. Furthermore, only a few matured trees of *V. yeechongii* have the ability to produce flowers and fruits. Apart from taxonomy, little is known about *V. yeechongii*. Understanding the flowering pattern, floral biology, breeding and pollination systems is important as it can fill in the gaps on the reproductive biology of *Vatica* species. These basic informations are essential and useful to be used for plan conservation management of endemic and endangered species.

1.2.1 Threats

The population of *Vatica yeechongii* in Selangor, Sungai Lalang Forest Reserve, Sungai Tekala Recreational Forest, 3° 03.485' N, 101° 52.373' E, alt. 79 m a.s.l (Saw, 2002) lies in an amenity forest. It is a protected area gazetted under National Forestry Act 1984 (NFA). Sungai Tekala Recreational Forest has been gazetted as water catchments for the Semenyih Dam to supply clean water for the community in Kuala Lumpur.

Sungai Tekala Recreational Forest is a very popular nature escape for outdoor activities ideally spent with families, perfect for short and weekend getaways. The slow and shallow river system meandering through the park is ideal for bathing and there are basic facilities available. The recreational forest offer activities such as camping, jungle trekking,

photography and bird watching. Sungai Tekala Recreational Forest is strategically located where it is not too far from the township area and easily accessible.

There are only seven individuals of *V. yeechongii* with diameter more than 2 cm found in Sungai Tekala Recreational Forest. *V. yeechongii* is found on gentle earth banks beside stream near the public campsite. As this area is designated for recreational purposes, clearing of the undergrowth and cleaning need to be done daily to maintain cleanliness. Even though the population lies in a protected area, human activities and regular cleaning will slowly affect the regeneration and survival of the population.

Another population of *V. yeechongii* in Negeri Sembilan, Setul Forest Reserve (2º 46.937' N, 101º 55.069' E) is located adjacent to the Mantin-Seremban main road. At the western side of the population's location, the new expressway Kajang-Seremban Highway (LEKAS) serves as effective traffic dispersal for the highly congested Kajang town in Selangor to Seremban in Negeri Sembilan besides the North-South Expressway Southern Route and the Kuala Lumpur-Seremban Expressway. It is of concern that this population may be threatened in the near future if road expansion is carried out to meet the needs of the growing traffic. The population is located in a very small isolated fragment forest margin, thus edge effects are likely to severely impact the population. The size of the fragment is estimated to be 2.0 hectare. There are only about 60 trees of *V. yeechongii* with diameter more than 1 cm occurring in Setul Forest Reserve, and the seedlings regenerate from year to year. The population in Setul Forest Reserve has been given a protection status under High Conservation Value Forest (HCVF), but there is no future guarantee in the population's viability. The forest fragment has no adequate buffer zone as it is surrounded by agricultural activities at the eastern side and main road on the western side. Previous studies showed that populations located closed to the borders of forest reserves suffered more threats against rural area in the forest reserve.

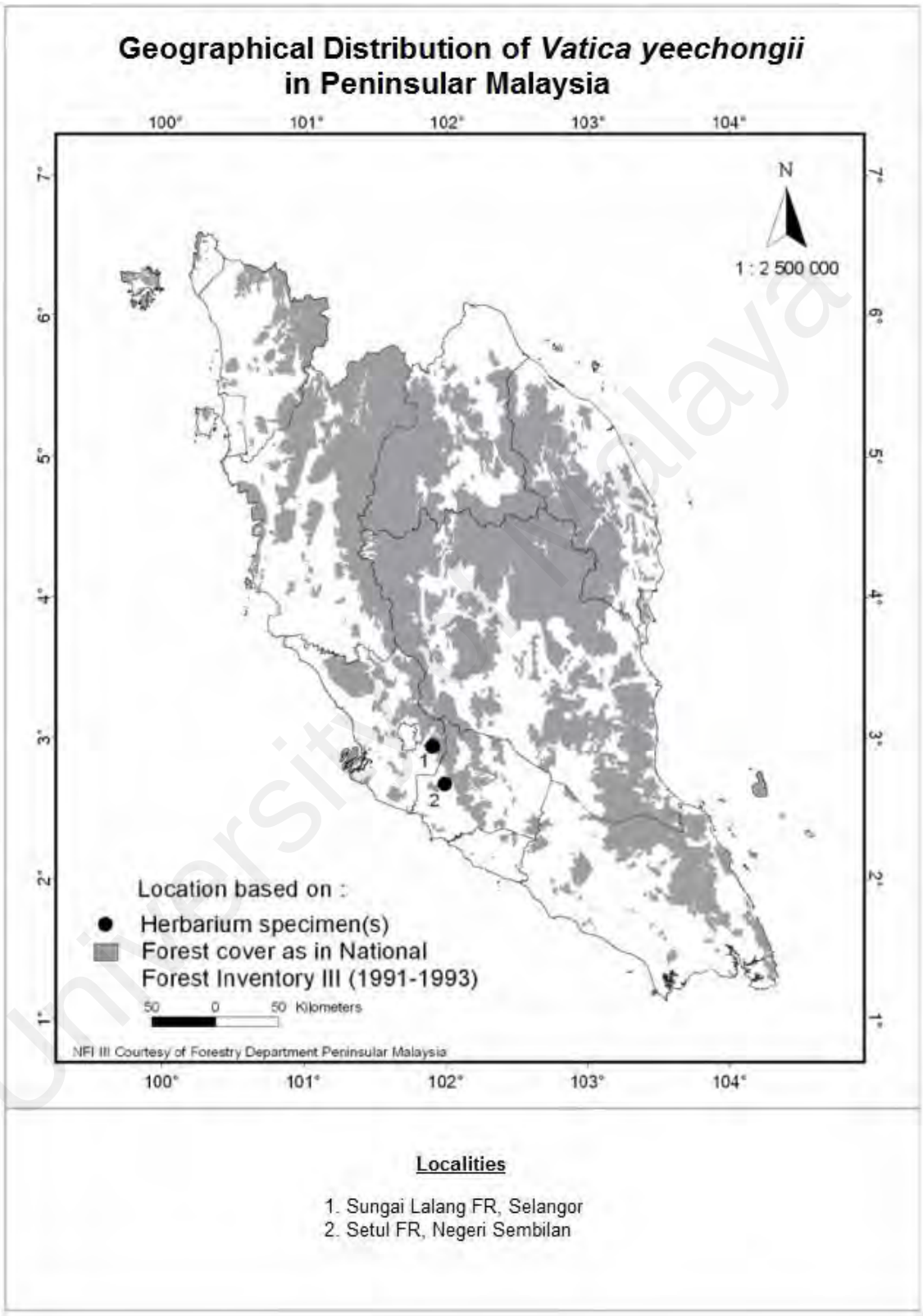

Figure 1.1: Localities of *Vatica yeechongii* in Peninsular Malaysia

1.2.2 Lack of studies

Studies on the reproductive biology of plants include addressing the right timing of recurring biological events such as bud formation and flowering, fruiting, pollination, breeding system and seed germination. Dipterocarpaceae is well known for it highest economic timber value. However, little information is known regarding its floral biology and breeding system. Previous studies on the reproductive biology of Dipterocarpaceae focused on the genus *Shorea* section Muticae (*Shorea leprosula*, *S. macroptera, S. acuminata, S. parvifolia, S. lepidota* and *S. dasyphylla*) and species under the genus *Dipterocarpus* (*Dipterocarpus globosus*, *D. geniculatus*, *D. obtusifolius* and *D. pachyphyllus*). Much of our understanding on the floral biology of the family including our assumptions for the genus *Vatica* is based on these works.

There is no detailed study on the reproductive biology of *Vatica* species. Out of the 15 species of dipterocarps categorized as Critically Endangered in Peninsular Malaysia, two are from the genus *Vatica* (Chua *et al.*, 2010). We question whether the flowering phenology, pollination and breeding system of *Vatica yeechongii*, an understorey dipterocarp, behaves in the same manner as *Shorea* and *Dipterocarpus*. More efforts should be given to research on the reproductive biology of *Vatica* species, as this approach allows us to understand more on the viability of a population and its gene flow, which are important for planning conservation management of rare species, as they are usually very restricted and habitat specific (Bawa *et al.*, 1985).

A High Conservation Value Forest (HCVF) has been declared for the population at Setul FR and the present study was aimed at understanding its floral phenology, breeding system and pollination biology. This information is crucial for predicting viability and survival and identifying factors in flowering and pollination that could potentially lead to bottleneck events. For the HCVF, this information is required to assist the development of management strategies aimed at effective conservation.

1.3 Objectives

It is the aim of the present study to understand and obtain more information by investigating the reproductive biology of *Vatica yeechongii*. Specific objectives of this study are:

1. To study the flowering and fruiting patterns of *Vatica yeechongii;*

2. To trace the phenological development of its flower and elucidate its breeding system;

3. To identify the potential pollinating agents of the species in their breeding systems;

4. To investigate how its reproductive behavior affects species conservation.

CHAPTER 2: LITERATURE REVIEW

2.1 Plant phenology

Plant reproductive biology addresses the biological events related to leaf flushing, bud formation and flowering, fruiting and seed germination. The right timing, duration and frequency are important to define these biological events such as flowering patterns or fruiting patterns of certain plant species. The periodicity or timing of recurring biological events related to short-term climate change is called phenology. The timing of these events can profoundly affect survival and reproductive success. Phenological events for plants involved flowering, fruiting, leaf flushing and seed germination (Leith, 1974)

Patterns of plant phenology are not only influenced by abiotic factors such as temperature and humidity, but also came from biotic factors such as herbivory, competition, pollination and seed dispersal. Low survivorship of seedlings (Tevis, 1958), low seed production (Augspurger, 1981), and high predation rates (Aide, 1992) occur when flowering, germination or leaf production happens at the wrong time. Insects or animals that predate the young leaves, mature or immature flowers, and fruits can greatly affect plant phenology (van Schaik *et al.,* 1993), and resource cycling in the forest. Many studies involving plant phenology have been conducted in tropical forests to describe resource availability for consumers (Murali & Sukumar, 1994; Morellato *et al*., 2000).

2.2 Flowering event

Members of the Dipterocarpaceae dominate the rain forests of Malaysia, comprising roughly 10% of all tree species, and up to 80% of canopy and emergent individuals (Ashton, 1982; Whitmore, 1998; Lee *et al.,* 2002). Dipterocarps have a unique rhythm in reproductive phenology known as mast flowering or general flowering, followed by mast fruiting (Ashton *et al*., 1988; Sakai 2002). The obvious difference between general flowering and other

masting phenomena is many other plant species also flowering synchronized with dipterocarp trees, not only among dipterocarp trees (Medway, 1972; Appanah, 1985; Sakai *et al*., 1999). Several dipterocarp and non-dipterocarp species flower simultaneously during general flowering events, which occur at irregular intervals of 2 to 10 years (Appanah, 1993).

Wood (1956) was the first person to describe in detailed about the mast flowering event. In 1955, he reported that more than two-thirds of the 200 dipterocarp species flowered and fruited in Sabah. Medway (1972) observed dipterocrap species in Gombak Forest Reserve and found 67% to 72% species flowered, respectively during the years 1963 and 1968. Appanah (1985) found over 70% of all dipterocarps flowered on flowering event in the 1981 at Pasoh Forest Reserve. Chan (1977) mentioned, besides *Shorea* species (section Muticae) studied at Pasoh in 1976, species from other genera of Dipterocarpaceae namely, *Dipterocarpus*, *Vatica*, and *Hopea* were also mast fruiting. He also mentioned that certain species belonging to Bombacaceae, Leguminosae, Burseraceae and Euphorbiaceae fruited alongside the Dipterocarpaceae.

During general flowering event, more than 80% of the plant species produces flower for about 4 or 5 months (Appanah, 1985; Ashton *et al*., 1988; Curran & Leighton, 2000; Numata *et al*., 2003). Appanah (1993) mentioned over 200 tree species come into heavy mass flowering and last for short period of 3 to 4 months. Wood (1956) recorded heavy flowering of non-dipterocarp species including native fruit trees during mass flowering in year 1955.

Sakai *et al.* (1999) concluded that the phenomenon mast flowering or general flowering takes place at the community level, which involved many plant species from many families. He studied and monitored reproductive phenology of 305 species from 56 families in Lambir Hills National Park, Sarawak. He also documented the first relatively complete cycle of plant reproductive phenology from one general flowering to another and classified

257 species into flowering types which is general flowering, supra annual, annual and sub-annual based on timing and frequency of flowering.

Sakai (2002) mentioned many meteorological factors involved in triggering the trees to flower during general flowering. The factors are temperature, rainfall, humidity and solar radiation which are closely related and it is difficult to identify the exact factors inducing general flowering. According to Sakai *et al.* (2006), drought is thought to be the factor that triggers initiating general flowering in the aseasonal tropical forests.

Based on meteorological records for 11 years, Ashton *et al.* (1988) suggested that a drop in the minimum temperature is the factor that triggers general flowering. Yasuda *et al*. (1999) mentioned about a drop of temperature one month before the onset of general flowering during his observation at Pasoh Forest Reserve in Peninsular Malaysia in 1996 and also at Lambir National Park in year 1996 and 1997. This hypothesis was supported by the evidence of low night-time temperature before flowering occurs (Ashton *et al*., 1988; Yasuda *et al*., 1999)

General flowering in Sumatran rain forest was found to have an association with hours of sunshine (Van Schaik, 1986). Decrease in solar radiation due to cloudiness might trigger the general flowering in Sarawak (Sakai *et al*., 1999). Dry seasons with monthly rainfall less than 100 mm occur within an annual cycle and many studies have shown strong correlations between rainfall and tropical plant phenology (Augspurger, 1981, Murali & Sukumar, 1994).

Estimations of flowering for *Shorea leprosula* through counting the fallen corolla for several trees, ranged from 63000 to 4000000 flowers per tree. The floral production of *Balanocarpus heimii* at Pasoh forest was estimated at 447000 (Chan, 1977). According to Harrison *et al*. (2005), *Dipterocarpus pachyphyllus* individual had approximately 450 flowers in total, which is smaller number of flowers than other *Dipterocarpus*.

2.3 Pollen study

Studies on pollen of the Dipterocarpaceae have been carried out by several researchers. Maury and Lugardon (1975) studied and documented including systematic descriptions and high resolution images of pollen of nine species from six genera of Dipterocarpaceae. Talip (2008) examined the pollen morphology of 32 species of Malaysian dipterocarps.

The shape of pollen gathered from herbarium specimens may change as a result of drying period and temperature (Price & Ayers, 2008). He recommended for using either FAA-preserved material collected in the field, or fresh pollen for morphological studies.

Penny *et al.* (2012) studied the pollen morphology using fresh pollen extracted from flowers for four dipterocarp species; *Parashorea tomentella, Shorea multiflora, Shorea xanthophylla* and *S. leprosula.* She mentioned pollen volume was positively correlated with flower size for the four species and concluded that pollen morphology could be used as a diagnostic tool for distinguishing dipterocarp species from different sections of genus and between genera.

Chan (1981) found that there are different sizes of pollen for *Shorea macroptera, S. lepidota, S. parvifolia, S. acuminata* and *S. leprosula* (section Muticae), which ranges between 0.0025 mm to 0.0030 mm. He also mentioned that *S. macroptera* produced most pollen per flower (5500/flower) compared to other four species in section Muticae that produced pollen varying from 3000 to 4250 per flower.

Ghazoul (1997) studied *Dipterocarpus obtusifolius* in dry decidous forests of Thailand and found that the species produced high number of pollen per flower, approximately 45000 with 6 ovules.

2.4 Breeding system

Chan (1981) reported that *Shorea macroptera, S. dasyphylla, S. lepidota, S. parvifolia, S. acuminata* and *S. leprosula* (section Muticae) are highly self-incompatible as there were greater success in fruit set derived from outcrossings than selfings. He also suggested that *Dipterocarpus obtusifolius* is self-compatible as fruit set from selfings is higher than crossings after one month of the compatibility test. Dayanandan *et al.,* (1990) mentioned that *Shorea megistophylla, S. cordifolia, S. congestiflora, S. trapezifolia* and *Vateria copallifera* in Sri Lanka appeared to be self-incompatible. Ghazoul (1997) suggested that *Dipterocarpus obtusifolius* in dry deciduous forests of Thailand are self-incompatible as there is wide spatial separation between the anthers and stigma in *D. obtusifolius* flowers.

Harrison *et al*. (2005) mentioned that *Dipterocarpus globosus* and *Dipterocarpus pachyphyllus* seem to have the capability of autogamy as both species produced fruit inside mesh bags. Bawa (1974) carried out controlled pollination experiments in a semi-deciduous forest in Costa Rica and found 54% and 22% of the tree species were self-incompatible and dioecous respectively, suggesting that outbreeding systems predominate.

Chan (1981) showed through controlled pollination experiments that *Shorea ovalis* had the possibility to be self-compatible. It is confirmed by Kaur (1977) that this species is apomictic based on embryological examinations. Kaur (1977) also provided evidence of apomictic species for *Hopea subalata, Shorea resinosa* and *S. macroptera.* Based on results from control baggings and open pollination, Chan (1981) suggested that *Hopea glabra* is either apomictic or self-compatible.

2.5 Pollinators

Understanding the symbioses between plant and pollinators is essential for sustainable management of the forest resources (Tani *et al.,* 2012). Differences in the flowering pattern may be related to the characteristics of their pollinators (Sakai *et al*., 1999).

Phenomenon of general flowering in dipterocarp forests is related to the existence of the staggered flowering of closely related species or those species sharing the same pollinators (Chan & Appanah, 1980; Appanah, 1993). Floral characters such as flowering time, rewards and floral shape were found to be significantly related to the pollination system of the dipterocarp species. However, floral color was however not significantly related (Momose *et al*., 1998).

Previous study showed that, *Shorea* species (section Muticae) adapted the pollination by energically-limited thrips. The thrips used the flowers as their breeding ground and as source of food (Chan & Appanah, 1980; Appanah & Chan, 1981). Kondo *et al*. (2016) investigated the pollinator of *Shorea curtisii* in a hill dipterocarp forest in Peninsular Malaysia and found that major visitors of *S. curtisii* in the hill dipterocarp forest were thrips and the predatory big-eyed bugs.

Large moths (Sphingidae and Noctuidae) were recognized as night pollinators of *Dipterocarpus obtusifolius* in the dry deciduous forests of Thailand, while butterflies (primarily Pieridae and Papilionidae) pollinated its flowers during the day (Ghazoul, 1997). Harrison *et al.* (2005) studied flowering phenology and pollination of seven *Dipterocarpus* species during general flowering in Lambir Hills National Park in 1996. He recorded five species; *Dipterocarpus crinitus, D. geniculatus, D. globosus, D. palembanicus* and *D. tempehes* were visited by large numbers of *Apis dorsata* and one species, *D. pachyphyllus* was visited by geometrid moths. He confirmed that the head and mouthparts of the moth carried the pollen.

Momose *et al.* (1998) studied the canopy and emergent species in Lambir Hills National Park and found that the flowers of two species of *Vatica*, two of *Hopea* and 17 species of *Shorea* were predominantly visited by small herbivorous beetles from the families Chrysomelidae and Curculionidae, while two species of *Dryobalanops* were visited by the giant honey bee, *Apis dorsata* F. (Hymenoptera, Apidae). Lee *et al.* (2016) postulated that

energetic and generalist pollinators such as social bees (*Apis* spp. and *Trigona* spp.) may be involved in the pollination of dipterocarps in the hill dipterocarp forests of Peninsular Malaysia.

CHAPTER 3: MATERIALS AND METHOD

3.1 Study sites

Two study sites have been selected for the phenological study, namely, Sungai Tekala recreational forest park which is located at Sungai Lalang Forest Reserve, Selangor (3º 03.485' N, 101º 52.373' E) and Setul Forest Reserve, Negeri Sembilan (2º 46.937' N, 101º 55.069' E). Sungai Tekala recreational forest park is a protected area gazetted for water catchment and recreational under National Forestry Act 1984 (Figure 3.1). The trees grow on earth banks beside a stream near the public campsite. Populations at Setul Forest Reserve, Negeri Sembilan grow in a very small isolated fragment forest margin of a logged-over forest, which is adjacent to the Mantin-Seremban main road. Scaffolding was erected under two trees at Sungai Lalang FR to access to the flowers (Figure 3.2).

Figure 3.1: Entrance of Sungai Tekala recreation forest park

Figure 3.2: Scaffolding erected under *Vatica yeechongii*

3.2 Methodology

3.2.1 Flower and fruit morphology

Flowers and fruits at different maturity stages were collected and dissected under a stereomicroscope. Flowers at anthesis were collected, fixed in FAA solution, dehydrated, coated and scanned under scanning electron microscope (Appendix A).

3.2.2 Flower and fruit development

As soon as the buds appeared on the branches, they were closely observed. The development of flower buds was observed and measured weekly. Fifteen inflorescences from three individual trees were observed. Direct observations were made to determine the time of flower opening, anther dehiscence and stigma receptivity. The floral parts were measured with digital calipers. To determine stigma receptivity, stigmas from the opening flowers were immersed in 3% of hydrogen peroxide (Carrington *et al*., 2003). If bubbling occurs, it indicates peroxidase activity and stigma was receptive.

At the end of flowering, development of fruit was observed. The diameter and length of fruits were measured weekly using digital calipers. The average increase in length and diameter were determined from matured fruits.

3.2.3 Flower and fruit phenology

Seven individuals in the population of Sungai Lalang FR and twenty five individuals in the population of Setul FR with DBH more than 3 cm were selected and observed for this study. The crown of the tree is individually scanned twice a week using binoculars according to Appanah and Chan (1982). One form per tree was used to record time, date of the observation, the intensity and stages of flowering and fruiting. The following categories were employed to describe the stages of flowering and fruiting:

Stages of flowering	Stages of fruiting
1 : budding	1 : fruits development
2 : initial bloom	2 : maturation
3 : peak bloom	3 : falling of matured fruits
4 : tail bloom	
5 : end of flowering	

The crown was divided into four sections and each of the sections was individually scanned. The intensity is gauged by the percentage of crown in flower or fruit (<25%, 25-50%, 50-75% and >75%). The information on flowering and fruiting intensity can be obtained by observing the fallen corollas or fruits.

The Malaysian Meteorological Department provided meteorological data, for year 2012 until 2014. Data of rainfall and minimum temperature were collected from the weather stations of Empangan Air Sungai Semenyih (N 2^{o} 56', E 101^{o} 52', 233.3 m above mean sea level, a.s.l.) and Hospital Seremban (N 2^{o} 43', E 101^{o} 56', 64.1 m above mean sea level, a.s.l.). The Empangan Air Sungai Semenyih was the nearest station to the study site at Sungai Lalang Forest Reserve, 2 km away while weather station Hospital Seremban is located about 8.5 km from the population in Setul FR.

3.2.4 Flower and fruit production

Floral and fruit production by an individual was estimated by counting the number of flowers and fruits produced on several selected branches to extrapolate for the whole tree. Three individuals of *Vatica yeechongii* were available for estimation of flower and fruit

production. The flower and fruit production was estimated according to the methodology identified by Appanah and Chan (1982), where

Total number of flowers/fruits = Number of flowering branches X average number of inflorescences per branch X average number of flowers per inflorescence

3.2.5 Breeding system

Three trees were selected for the pollination experiments to determine the breeding system of this species. The criterion for selection of tree depends on the availability and accessibility of flowering branches. Five treatments were applied, each with 60 replicates:

i. Control for selfing : Flowers bagged with no manipulations
ii. Open-pollination : Inflorescences tagged without manipulations
iii. Self-pollination (Geitonogamy) : Flowers emasculated, bagged and pollen from other flowers of the same tree applied
iv. Cross-pollination (Xenogamy) : Flowers emasculated, bagged and pollen from another tree applied
v. Without pollination (Emasculation) : Flowers emasculated and bagged

The inflorescences with matured flower buds to be used in cross-pollination, self-pollination and emasculation were emasculated prior to anthesis and then bagged by using organza mesh size 0.2 mm. Prior to control for selfing, cross-pollination, self-pollination and emasculation, any open or immature flowers were removed from the inflorescences.

In the process of emasculation, all stamens were carefully removed by using a pair of fine forceps and pollen was applied with a fine tip brush. In this case, different brush were used for each treatment to ensure that it was free from pollen grains. The flowers were kept bagged for two weeks. Fruit set was monitored and counted. The treatments were considered successful when the fruit nut reached mature fall stage.

The index of self-incompatibility (ISI) for *Vatica yeechongii* was determined using the formula proposed by Zapata and Arroyo (1978), where

$$ISI = \frac{\text{Fruit set from self-pollination}}{\text{Fruit set from cross-pollination}}$$

The values of the ISI correspond to the breeding system as such:

>1	self compatible
>0.2<1	partially self-incompatible
<0.2	mostly self-incompatible
0	completely self-incompatible

3.2.6 Potential pollinators

Casual observation was done on flower visitors. The observations for visitors were conducted on 16 March 2014 until 20 March 2014. Samples collection started from 0600 to 1200 hours from three flowering trees. Detailed observations were made on 17 March 2014 by counting the frequency of occurrence of the insects for five minutes at 30 minutes interval.

The visitors were caught using hand nets and stored in 90% alcohol. Small flower visitors such as thrips were caught by collecting the flowers into small plastic vials and the thrips isolated from the flowers. Length of visits, feed characteristics, flight and search pattern of visible visitors were recorded. Where possible the insects were identified up to

genus and species level. The identification of insect specimens were done by expertise from FRIM and UKM. Specimens were observed under Scanning Electron Microscope (SEM) for the presence of pollen grains on their bodies. The presence of pollen grains on the bodies was compared with reference specimens of pollen from *Vatica yeechongii* flowers.

3.2.7 Pollen to ovule ratio

The ratio of pollen grains to ovules (P:O) in flower is to indicate the breeding system of the species (Cruden, 1977). The P:O ratio is calculated by estimating total pollen grains per flower and divided by number of ovules in a flower. All undehisced anther in a flower were collected with care using forceps to avoid the loss of pollen and transferred to a clean concave slide containing a drop of detergent and water. The anthers were squashed and the slide viewed under a light microscope to calculate the number of pollen grains per anther. The average number of pollen grains per anther was multiplied by the total number of anthers in a flower to get the total number of pollen grains per flower. The number of ovules was directly counted by dissecting the carpel of the flower.

3.2.8 Pollen morphology

Pollen was collected from newly opened flowers after anthesis and dried at 40^{O}C. They were then acetolysed, stained and mounted onto slide in Safranin-glycerine jelly (Appendixes B). The length (i.e polar axis) and diameter (i.e equatorial axis) of acetolysed and untreated pollen grains were measured using the Leica QWin Image Processing and Analysis System. The ratio of the pollen length (P) to its diameter (E) was calculated to determine the pollen shape. The shape and surface of acetolysed and untreated pollen was observed under scanning electron microscope.

3.2.9 Pollen germination

The pollen grains collected from newly opened flower were used for germination test in sucrose solutions. The pollen was cultured using the sitting drop technique (Shivanna & Rangaswamy, 1992) for three hours at room temperature (26-29 ^{o}C) (Appendixes C). Seven different media were used: distilled water, 5, 10 and 15%, each with 0.01% boric acid added, and 5, 10 and 15% sucrose solutions without boric acid (b.a).

The cultures were examined for germination from time to time. After three hours, the cultures that showed germination were each added a drop of formalin-acetic acid-alcohol (FAA) and scored under a microscope. The pollen grains were considered germinated when the length of its tube is equal to, or greater than the diameter of the pollen grains. The media that showed favourable germination (i.e. 10% and 15% sucrose solutions with 0.01% boric acid) were used in subsequent replicates. Pollen grains aged 2, 4, 8, 14, 27 and 42 hours after anthesis were used in subsequent replicates and scored after three hours. The length of 20 pollen tubes from each medium was measured using Leica QWin Image Processing and Analysis System.

3.2.10 Seed germination

The matured fruits was collected and sown to determine the percentage of germination. Three media were used; sand, recreational park soil and sand mixed recreational park soil (mixed in the ratio of 2:1). The morphology of seedlings such as germination type, colour and length of cotyledon and time taken to germinate were observed and recorded.

CHAPTER 4: RESULTS

4.1 Flower and fruit morphology

Vatica yeechongii is categorized as small tree within the family Dipterocarpaceae. It can reached up to 18-19 m high with diameter at breast high (DBH) c. 9-13 cm (Figure 4.1). The bark is smooth with greyish white colour and has horizontal rings. It has slightly drooping branches and robust twigs. One of the main characters of *Vatica yeechongii* is its large size leaves about 40-90 x 9-17 cm, with 28-30 pairs secondary veins. The leaf blade is oblanceolate with acuminate apex and thickly coriaceous.

The flowers are creamy white with a funnel shaped of corolla (Figure 4.2). It has five sepals and five oblong petals twisted with size of petal about 17.0 x 7.0 mm which is overlapping or alternate at the base and much longer than sepals (Figure 4.3, Figure 4.4). Pedicels 2-4 mm in length are light brown with densely rufous stellate hairs.

The flower has fifteen stamens with about the same length ca. 1.3 mm. The stamens are short, creamy white in colour and arranged in two whorl at the base of the flower with 5 and 10 stamens per whorl respectively (Figure 4.5). The arrangements of stamens are slightly curved, turned inward toward the axis. The filaments are very short and broader at the base with anther attached to the apex. The anthers are creamy white, oblong and basifixed (Figure 4.6). The anthers dehisced along a longitudinal line releasing large amount of creamy white pollens (Figure 4.7).

The ovary is creamy white, ovoid in shape, superior and glabrous or hairy. The style is macrostylous measuring 1.1 mm to 1.3 mm and attached to the centre of the ovary (Figure 4.8). The stigma is positioned higher than the anther (c. 0.8 mm at vertical distance). The measurements of floral parts are summarized in Table 4.1.

The fruit has five calyx measuring 33-42 x 1.4-2.0 cm. The young fruits are green at the base and reddish towards the tip (Figure 4.9), or fully reddish (Figure 4.10) and they turn

reddish brown or dark brown at maturity. The nut is ovoid with 14.0-17.5 cm diameter. The nut is light brown when young then turn pale yellowish green when matured with pointed tip (Figure 4.11). The nut is completely hidden inside the calyx lobes, but not directly attached to the calyx.

Figure 4.1: *Vatica yeechongii,* habit

Figure 4.2: Flowers with a funnel shaped corolla

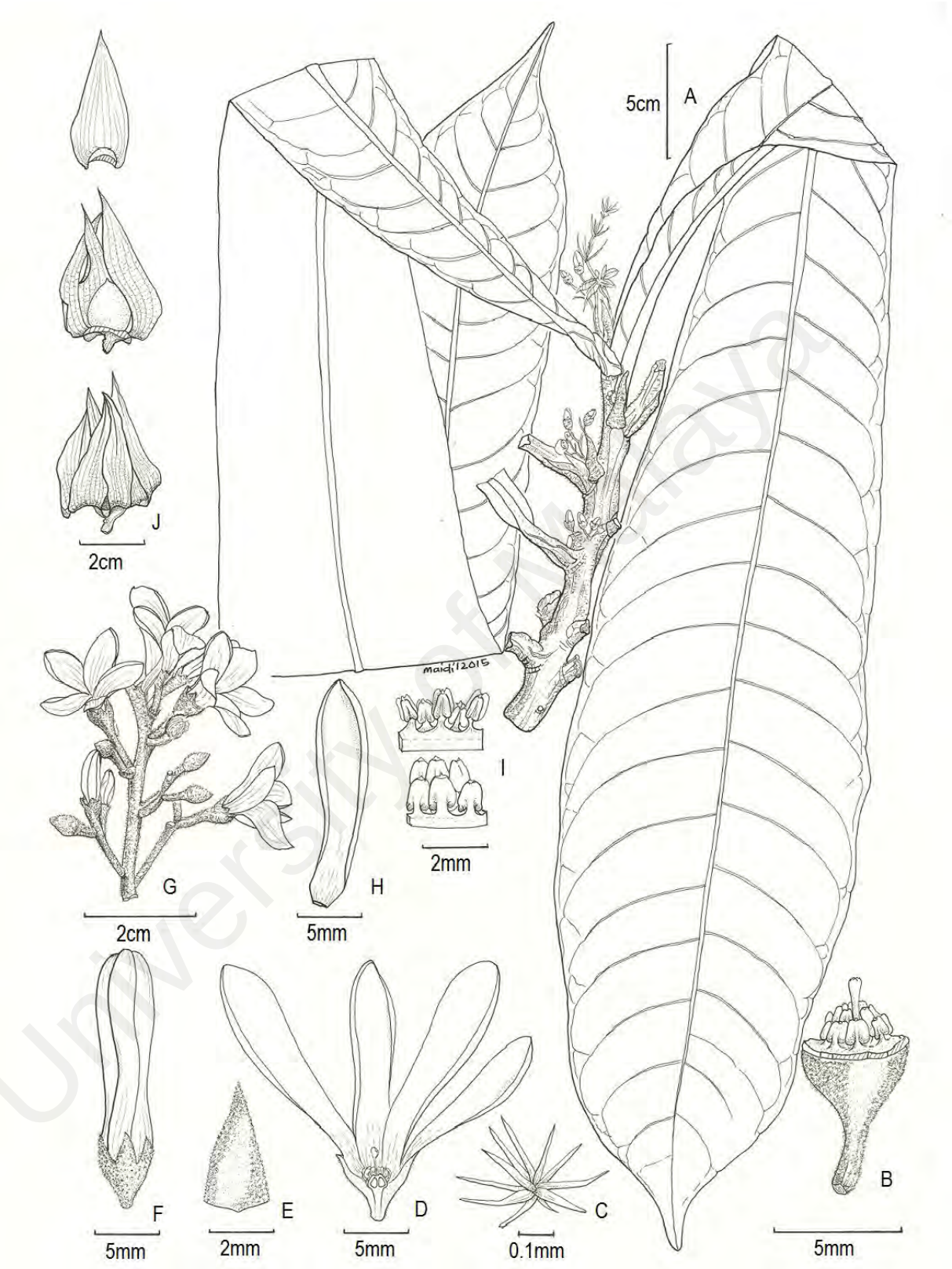

Figure 4.3: Floral morphology of *Vatica yeechongii*. A. Flowering leafy twig. B. Gynoecium. C. Stellate hair. D. Longitudinal section of open flower. E. Sepal. F. Flower bud. G. Inflorescences H. Petal. I. Anthers. J. Fruit, side view (bottom), nut of fruit (middle), calyx of fruit (top)

Figure 4.4: The flower has five oblong petals twisted

Figure 4.5: The flower has fifteen stamens

Figure 4.6: The flower has oblong and basifixed anthers

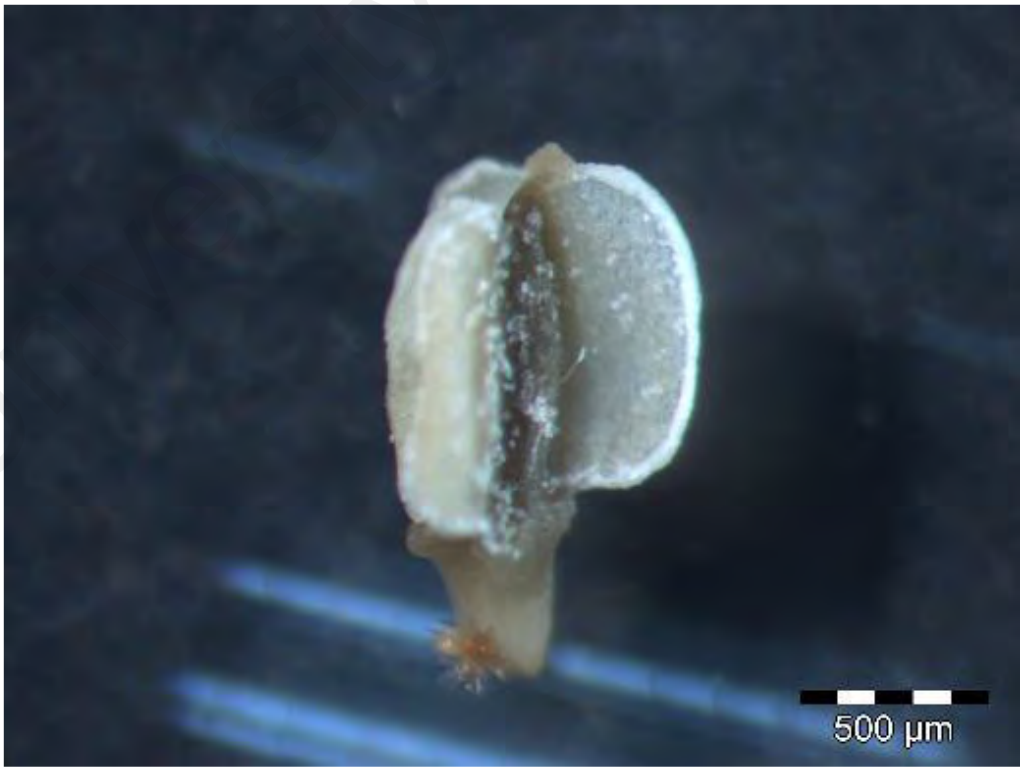

Figure 4.7: Anther dehiscence occurs along the longitudinal slits

Figure 4.8: The style attached to the centre of the ovary

Figure 4.9: The colour of young fruit is green at the base and reddish towards the tip

Figure 4.10: The colour of mature fruit is reddish brown or dark brown

Figure 4.11: The mature nut inside the calyx lobes

Table 4.1: Measurements of the floral parts

Flower parts	Length (mean + SD)	Sample size (No. of samples)
Diameter of open flower	21.02 ± 1.78 mm	20
Mature bud prior anthesis	20.39 ± 0.76 mm	23
Stamen	1343.61 ± 111.90 µm	15
Anther	1055.89 ± 57.99 µm	15
Style	1209.05 ± 50.90 µm	15
Pistil	2604.26 ± 145.06 µm	15
Petal	1.78 ± 0.12 cm	15
Inflorescences	6.54 ± 2.43 cm	15

4.2 Flower and fruit development

4.2.1 Floral biology

Flower of *V.yeechongii* started to open at early dawn around 0530 hour (Figure 4.12). The flowers are creamy white in colour and the diameter of opened flower is about 17.0 mm to 23.0 mm. They exuded a strong sweet scent which attracted insect pollinators. Generally, all the flowers started to open more or less simultaneously and the opened flowers remain open for about four to five hours (Figure 4.13).

The anthers of *V. yeechongii* started to dehisce upon floral opening. The anthers dehisce longitudinally and release white pollen grains. A portion of pollen grains is shed onto the petals. The stigmas become receptive 30 minutes later after the flower has opened and remained so for about two to three hours. Dehiscence of petals and anthers began as early 0930 hours (Figure 4.14).

Figure 4.12: Flower of *V.yeechongii* started to open at early dawn

Figure 4.13: Fully opened flower

Figure 4.14: Dehiscence of petals and anthers

4.2.2 Flower and fruit development

A new development of inflorescence could only be noticed when inflorescence bud appeared on the branch (Figure 4.15). The inflorescence buds are rigid with light brown colour, densely rufous stellate hairs on it. The inflorescence buds were tagged before the length reached 3 mm. After 4-7 days, the bud breaks, exposing the inflorescence. The young flower buds appeared and completely formed approximately 10-14 days after inflorescence initiation (Figure 4.16).

During the subsequent expansion of the inflorescences, young flower buds continued to grow from green bud into creamy white matured flower buds (Figure 4.17). These inflorescences have brown colour with rufous stellate hairs on it. The number of flowers per inflorescence varies, ranging from 1 up to 56 flowers (mean = 23.40, SD = 11.73, N inflorescence = 30) (Figure 4.18). The length of the inflorescences is between 2.0 cm to 10.0 cm (mean = 6.54, SD = 2.43, N inflorescence = 15).

The flower of *V. yeechongii* grows in a sigmoid-curve pattern (Figure 4.19). The initial growth was slow, but increased rapidly after 10 days. The matured flowers buds open synchronously. The growth of flower bud from the inflorescence initiation required between 26 to 32 days.

By the third week after a successful pollination, young fruits begin to develop and increase in size. The young fruits have 5 calyx, with green colour and red at the tip that grows up from sepal of the flower and small light brown nut resides in the middle of the calyx. The calyx grows faster compared to the nut. The nut enlarges and changes in colour from light brown to light green after 60 to 70 days. The calyx of the fruit turns reddish green and some of it turn reddish brown when matured, while the nut turn yellowish green.

The fruits grow in a sigmoid curve pattern (Figure 4.20). The growth was slightly slow at the beginning, before increasing steadily and finally slowed down until matured stage. The length and diameter of the fruits of *Vatica yeechongii* grow in equal proportions.

The growing process of young fruits until falling of matured fruits takes about 80 to 90 days (Figure 4.21). Most young fruits abort at early stage of development. Usually only 1-5 fruits per infructescence managed to reach matured stage. More often, the whole infructescence fails to bear any fruits. Some of the fruit did not reach matured stage as it has been eaten by monkeys and squirrels. Generally, all fruits within an infructescence reached maturity more or less simultaneously.

Figure 4.15: The appearance of inflorescence buds on the branch

Figure 4.16: The young flower buds

Figure 4.17: The matured flower buds

Figure 4.18: The inflorescence

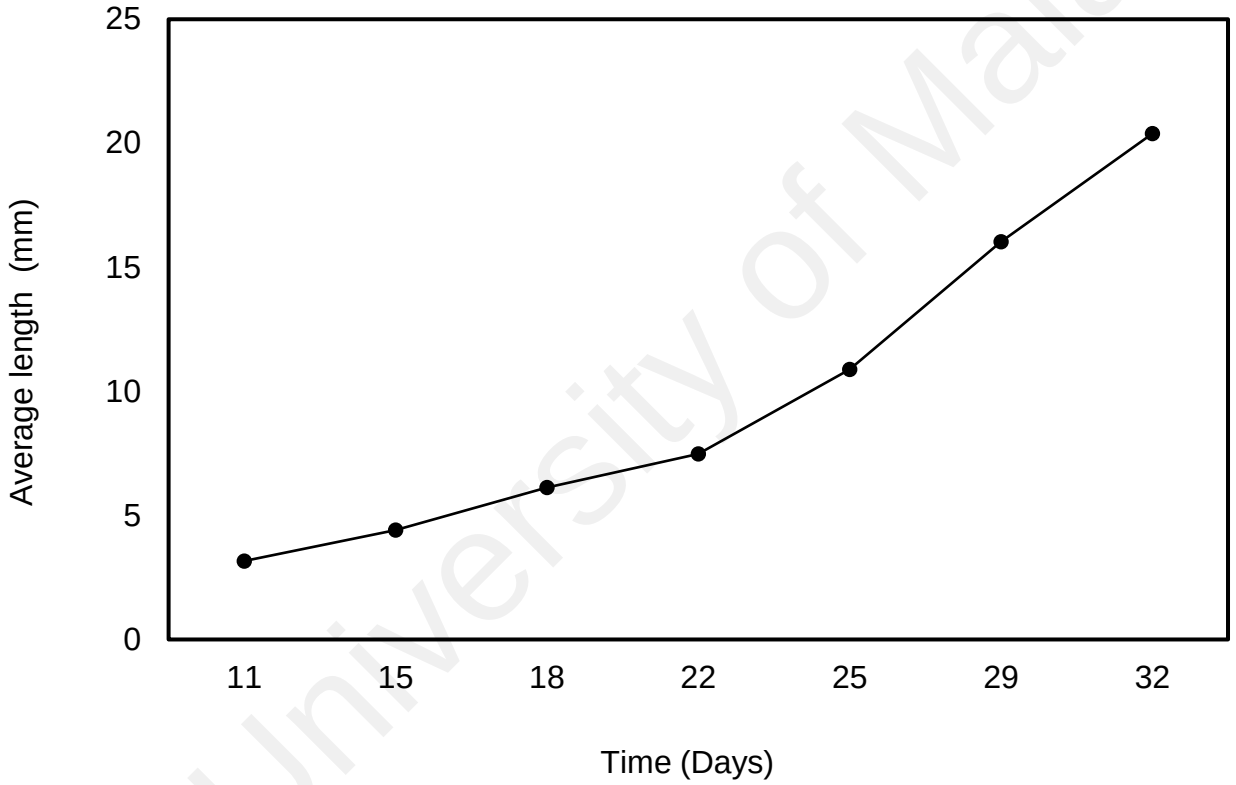

Figure 4.19: Average length of flower development in *Vatica yeechongii*

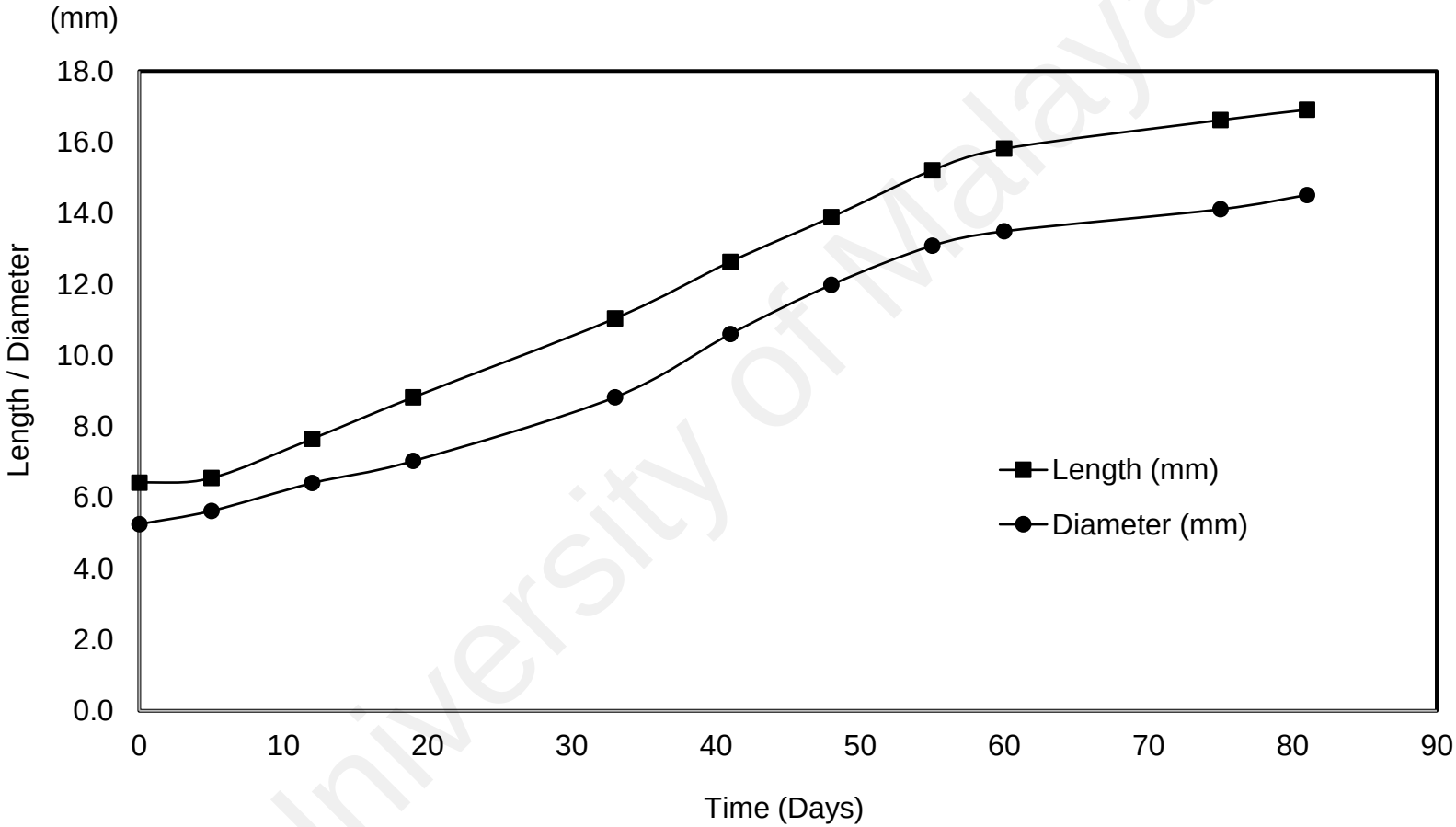

Figure 4.20: Average length and diameter of fruit development in *Vatica yeechongii*

Figure 4.21: Young fruits of *Vatica yeechongii*

4.3 Flower and fruit phenology

4.3.1 Phenology at the population level

Based on three years observation that been done starting from January 2012 until December 2014, trees of *Vatica yeechongii* in both populations produce flower every year. There were trees with the capability to produce flower twice a year. Each tree also had different intensity of flowering at each flowering periods (Table 4.2). Observations in February 2012 recorded five trees in Sungai Lalang FR and four trees in Setul FR started to develop flowers. In year 2013, only few trees from both populations produced flowers starting from March 2013. All seven trees of *V. yeechongii* observed at Sungai Lalang FR and ten trees at Setul FR started to develop flowers simultaneously in February 2014.

The flowering period in year 2014 was the most successful as both populations recorded the highest number of flowering trees compared to year 2012 and 2013. There were trees in both populations not flowering in year 2012 and 2013, but flowering in year 2014. There were only two trees at Sungai Lalang FR and four trees at Setul FR producing flowers every year.

First cycle period of flowering in both populations started in February 2012, March 2013 and February 2014, and the trees fruited profusely. In the second period of flowering in September 2012, November 2013 and October 2014, the trees resulted in little or no fruit set. The duration of flowering to mature fruit fall was 21-22 weeks.

Each tree also had different intensity of flowering at each flowering periods (see Tables 4.3 and 4.4). The first cycle of flowering period in year 2014 was the most successful flowering period at Sungai Lalang FR when five individual trees had more than 75% of crown in flower, one tree with 50-75% and one tree with 25-50%. Population of *V. yeechongii* at Setul FR also flowered gregariously in 2014 when five individual trees had more than 75%, four trees with 50-75% and one tree with 25-50% of crown in flower. Different situation happened in year 2013 when trees had low flowering intensity in both populations

as there were only two and four trees flowered with <25% flowering intensity, recorded from Sungai Lalang FR and Setul FR, respectively. In year 2012, population at Sungai Lalang FR recorded three individuals had more than 75%, two individuals with <25% flowering intensity and another two not flowering. While at Setul FR, four individuals with <25% of crown in flower and twenty one not flowering.

In the second flowering period, none of the trees at Sungai Lalang FR had flowering intensity more than 25%. The highest number of flowering in second period recorded at Sungai Lalang FR when three individuals with mean intensity <25% of crown in flower which happened in year 2012. In year 2013 and 2014, only two trees flowering with intensity <25% of crown in flower. From the observations, only three individuals at Sungai Lalang FR had capability to produce flowers twice a year, while trees at Setul FR only flowered once a year.

Table 4.2: The number of flowering/fruiting individuals and the range of flowering/fruiting intensity during first period (February–March) and second period (September-November) of flowering/fruiting (in parenthesis; 1=<25%, 2=25-50%, 3=50-75% and 4=>75%, of the crown)

Locality	No. tree observed	No. tree flowering/fruiting		
		2012	2013	2014
Setul FR	25	4, 2 [a](1), (1) [b](1), (1)	4, 0 [a](1), (0) [b](1), (0)	10, 0 [a](2-4), (0) [b](1-3), (0)
Sungai Lalang FR	7	5, 3 [a](1-4), (1) [b](3), (1)	2, 2 [a](1), (1) [b](1), (0)	7, 2 [a](2-4), (1) [b](1-3), (1)

Notes: a=flowering intensity; b=fruiting intensity

Table 4.3: The intensity of flower and fruit of *Vatica yeechongii* at Sungai Lalang FR

		Year											
		2012				2013				2014			
Tree No.	**DBH (cm)**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**
1	18.5	>75%	Feb	50-75%	Jul	-		-		>75%	Feb	50-75%	Jul
2	29.9	>75%	Feb	50-75%	Jul	-		-		>75%	Feb	50-75%	Jul
3	14.5	>75% <25%	Feb Sept	50-75% -	Jul	<25% <25%	Mar Nov	<25% -	Aug -	>75% <25%	Feb Oct	50-75% -	Jul
4	9.9	-		-		-		-		50-75%	Feb		Jul
5	9.7	<25% <25%	Feb Sept	- <25%		- -		- -		>75% -	Feb	- 	Jul
6	18.8	<25% <25%	Feb Sept	- <25%	Feb 2013	<25% <25%	Mar Nov	<25% -	Aug -	>75% <25%	Feb Oct	<25%	Jul Feb 2015
7	3.1	-		-		-		-		25-50%	Feb		Jul

(-) * Tree not producing flower / mature fruit

Table 4.4: The intensity of flower and fruit of *Vatica yeechongii* at Setul FR

		Year											
		2012				2013				2014			
Tree No.	**DBH (cm)**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**
1	12.4	<25%	Feb	<25%	Jul	<25%	Mar	<25%	Aug	50-75%	Feb	25-50%	Jul
2	14.2	<25%	Feb	<25%	Jul	<25%	Mar	<25%	Aug	>75%	Feb	50-75%	Jul
3	4.8	-		-		-		-		-		-	
4	3.8	-		-		-		-		-		-	
5	20.1	-		-		-		-		>75%	Feb	50-75%	Jul
8	15.5	-		-		-		-		-		-	
9	3.1	-		-		-		-		-		-	
10	4.6	-		-		-		-		-		-	
11	14.0	<25%	Feb	<25%	Jul	-		-		-		-	
12	9.4	-		-		-		-		50-75%	Feb	<25%	Jul
13	3.4	-		-		-		-		-		-	
14	5.6	-		-		-		-		25-50%	Feb	<25%	Jul
15	14.4	-		-		-		-		>75%	Feb	50-75%	Jul
16	3.0	-		-		-		-		-		-	

Table 4.4, continued

		Year											
		2012				2013				2014			
Tree No.	**DBH (cm)**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**	**Intensity of flower**	**Month**	**Intensity of fruit**	**Month**
17	3.4	-		-		-		-		-		-	
18	4.2	-		-		-		-		-		-	
19	4.4	-		-		-		-		-		-	
21	32.1	<25%	Feb	<25%	Jul	-		-		>75%	Feb	25-50%	Jul
23	4.0	-		-		-		-		-		-	
24	10.2	- <25%	Jul	- <25%	Dis	<25% -	Mar	<25% -	Aug	50-75% -	Feb	50-75% -	Jul
25	3.2	-		-		-		-		-		-	
27	14.1	- <25%	Jul	- <25%	Dis	<25% -	Mar	<25% -	Aug	>75% -	Feb	 -	Jul
28	7.5	-		-		-		-		-		-	
30	6.3	-		-		-		-		-		-	
31	12.8	-		-		-		-		50-75%	Feb	<25%	Jul

(-) * Tree not producing flower / mature fruit

4.3.2 Flowering behaviour

Detailed phenological data on *Vatica yeechongii* trees was collected during flowering event in year 2014 (see Table 4.5). All individuals were observed to flower profusely and synchronously. Flowers of *V. yeechongii* took much longer time to develop from the initial of young buds into bloom flower, approximately 5 weeks.

Based on the observation, the trees showed some variations in flowering behaviour. Tree no.7 has the smallest DBH which is 3.1 cm and had a late start in budding followed by an early termination of bloom. It also had the shortest flowering period. Meanwhile, tree no.6 had early initial bloom but had prolonged finishing phase which took much longer time to reach termination of bloom. This might be due to its location close to the river and part of the roots immersed in the river. Thus, the tree obtained enough water resources compared to other trees and induced the tree to continue producing flowers. Both trees no.1 and no.2, had synchronized flowering stages starting from budding until reach termination of bloom. All seven individuals had the same time of peak bloom stage.

4.3.3 Fruiting behaviour

From the observation, all seven flowering individuals produced mature fruit set (see Table 4.6). The period from the end of flowering to fruit ripening for *Vatica yeechongii* was approximately 14 to 15 weeks. Most young fruits or unsuccessful flowers in pollination abort in developing stage which is 4 or 5 weeks after end of flowering. These species took about 6 to 7 weeks in developing process from end of flowering into mature fruits. Mature fruit fall occurred during the third and fourth week of July.

Table 4.5: Flowering behavior of seven individuals of *Vatica yeechongii* at Sungai Lalang FR in year 2014

	Date of observations									
Tree No.	**6/2**	**13/2**	**19/2**	**28/2**	**6/3**	**10/3**	**12/3**	**16/3**	**18/3**	**20/3**
1	1	1	1	1	1	1	2	2	3	3
2	1	1	1	1	1	1	2	2	3	3
3	1	1	1	1	1	1	2	2	3	3
4	-	1	1	1	1	1	1	1	2	2
5	-	1	1	1	1	1	1	1	2	3
6	1	1	1	1	1	1	2	2	3	3
7	-	1	1	1	1	1	1	1	1	2

	Date of observations								
Tree No.	**23/3**	**25/3**	**27/3**	**31/3**	**2/4**	**5/4**	**10/4**	**11/4**	**15/4**
1	3	3	3	4	4	4	5	-	
2	3	3	3	4	4	4	5	-	
3	3	3	3	4	4	4	4	5	-
4	3	3	3	4	4	4	4	5	-
5	3	3	3	4	4	4	5	-	
6	3	3	3	4	4	4	4	4	5
7	2	3	3	4	4	4	5	-	

Table 4.6: Fruiting behavior of seven individuals of *Vatica yeechongii* at Sungai Lalang FR in year 2014

	Date of observations								
Tree No.	**17/4**	**28/4**	**5/5**	**19/5**	**28/5**	**3/6**	**10/6**	**16/6**	**25/6**
1	1	1	1	1	1	1	2	2	2
2	1	1	1	1	1	1	2	2	2
3	1	1	1	1	1	1	2	2	2
4	1	1	1	1	1	1	2	2	2
5	1	1	1	1	1	1	2	2	2
6	1	1	1	1	1	1	2	2	2
7	1	1	1	1	1	1	2	2	2

	Date of observations			
Tree No.	**2/7**	**9/7**	**16/7**	**23/7**
1	2	2	3	
2	2	2	3	
3	2	2	2	3
4	2	2	3	
5	2	2	2	3
6	2	2	3	
7	2	2	2	3

4.3.4 Correlation of flowering trees with rainfall and temperature

Vatica yeechongii flowered annually during the dry season where the trees began to produce flower buds in February and March 2014. February 2014 was the driest month at Setul FR as total rainfall recorded is 1.0 mm, and coincidently 10 from 25 trees observed produced flowers. While at Sungai Lalang FR, all seven trees also start to develop flowers simultaneously in February 2014 with total rainfall recorded at 18.60 mm.

There was almost similar pattern in the mean minimum temperature from year 2012 until 2014 at both weather stations (Figure 4.22). The highest total rainfall per year recorded in Empangan Air Semenyih is 2012, 3519.2 mm of rainfall recorded, then 2755.3 mm in 2013 and 2129.1 mm in 2014. While for Seremban Hospital, 2870.1 mm was the highest total rainfall recorded in 2014, 2560.2 mm and 2387.8 mm total rainfall in 2012 and 2013, respectively.

Within 36 months of observations, starting January 2012 until December 2014, the highest total rainfall per month or the wettest month for both stations was recorded in November 2012. The driest month which is the lowest rainfall recorded for station Empangan Air Semenyih was in September 2014 (4.6 mm) and for station Seremban Hospital was in February 2014 (1.0 mm). Average rainfall for 36 months period is 233.4 mm for station Empangan Air Semenyih and 217.2 mm for station Seremban Hospital.

Both weather stations recorded the highest mean of minimum temperature in June 2014 with 23.53°C and 25.09°C for Empangan Air Semenyih and Seremban Hospital, respectively. While, the lowest mean of minimum temperature for Empangan Air Semenyih is recorded in November 2014 (21.17°C) and for Seremban Hospital was in December 2014 (22.77°C). The average mean of minimum temperature for 36 months was 22.7°C and 23.7°C for Empangan Air Semenyih and Seremban Hospital, respectively.

Correlating the number of flowering trees with monthly rainfall and mean of minimum temperature, it did not show satisfactory correlation. Based on the Spearman's Rho

Correlation analyses (two-tailed) showed that no significant correlation between the number of flowering trees at Sungai Lalang FR and Setul FR with the mean of minimum temperature (with respective, correlation coefficient, $\rho = 0.011, -0.019$; P-value = 0.949, 0.910; n = 36, 36). For population of *V. yeechongii* at Sungai Lalang FR and Setul FR, there was no significantly correlation between the number of flowering trees with the rainfall too (with respective, $\rho = -0.136, -0.113$; P-value = 0.430, 0.513; n = 36, 36). (Appendix 1).

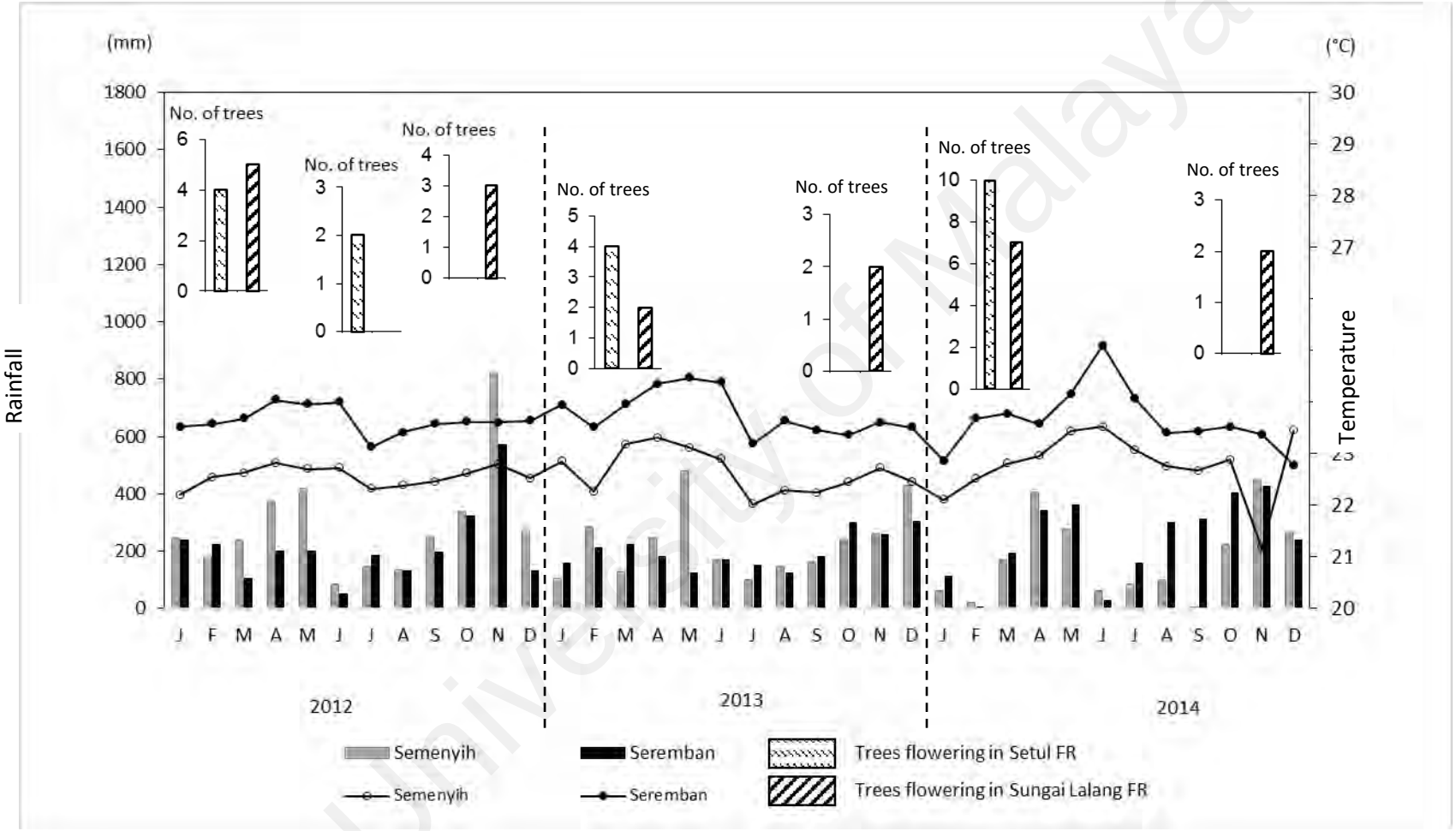

Figure 4.22: Records of rainfall (bar graph) and mean minimum temperature (line graph) from the weather stations in the Empangan Air Semenyih and Seremban Hospital with trees flowering in Setul FR and Sungai Lalang FR

4.3.5 Flower and fruit production

Only three individuals were studied for estimation of floral production. These three individuals were selected based on the accessibility of the tree as other trees were located on the river banks and difficult to observe. Floral production by three individuals of *V. yeechongii* from 2012 until 2014 is given in Table 4.7.

Observations on three individuals of *V. yeechongii* showed there are variation in flowering behaviors and number of flowers produced every year. All three individuals not flowering in 2013, but flowered gregariously in 2014. Trees no.4 and no.7 had massive number of flowers after two years of resting period, which is 756 and 448, respectively in 2014. Tree no.5 that had about 420 flowers in 2012, managed to produce about 4048 flowers in 2014.

As for fruit production, tree no.5 produced 300 fruits in 2014 and 9 fruits in 2012. Meanwhile, tree no.7 produced small number of fruits (about 16) in 2014 (see Table 4.8).

Table 4.7: Estimation of flower production for three individuals of *V. yeechongii* at Sungai Lalang FR

	Year		
	2012	**2013**	**2014**
Tree no.4			
Number of flowering branches per tree	not flowering	not flowering	7
Average number of inflorescens per branch			9
Average number of flowers per inflorescence			12
Total number of flowers			756
Tree no.5			
Number of flowering branches per tree	2	not flowering	11
Average number of inflorescens per branch	14		23
Average number of flowers per inflorescence	15		16
Total number of flowers	420		4048
Tree no.7			
Number of flowering branches per tree	not flowering	not flowering	2
Average number of inflorescens per branch			16
Average number of flowers per inflorescence			14
Total number of flowers			448

Table 4.8: Estimation of fruit production for three individuals of *V. yeechongii* at Sungai Lalang FR

	Year		
	2012	**2013**	**2014**
Tree no.4			
Number of fruiting branches per tree	not fruiting	not fruiting	7
Average number of infructescence per branch			6
Average number of fruit per infructescence			3
Total number of fruits			126
Tree no.5			
Number of fruiting branches per tree	1	not fruiting	10
Average number of infructescence per branch	3		10
Average number of fruit per infructescence	3		3
Total number of fruits	9		300
Tree no.7			
Number of fruiting branches per tree	not fruiting	not fruiting	2
Average number of infructescence per branch			8
Average number of fruit per infructescence			1
Total number of fruits			16

4.4 Breeding system

4.4.1 Pollination experiments

Three trees were selected for compatibility studies. Selection of trees was based on the availability and accessibility of inflorescences for pollination. Scaffolding was setup next to the tree as a way to access to the flowers. As accessible to the tree branches are limited, only small number of pollination experiments could be done.

In open pollination, only 28.3% which is less than half of the flowers had a successful fruit set. Flowers that were emasculated and then bagged also had unsuccessful fruit set (see Table 4.9). This showed that pollination was necessary to produce fruits and apomixis did not occur in *V. yeechongii.*

Flowers that were bagged without any manipulations for control treatment did not produce any fruit. In self-pollination where flowers were emasculated, pollen from flower of the same tree applied and bagged did not develop mature fruits. This indicated that *V. yeechongii* was self-incompatible to produce fruits.

In cross-pollination where pollens from flowers of different tree was applied, 13.3% of treated flowers managed to produce fruits (Figure 4.23). From the results, it is suggested that *Vatica yeechongii* is an outcrossing species. Besides, pollinating agents were required to transfer the pollen from anthers to the active stigma of *V. yeechongii*.

According to Zapata and Arroyo (1978), the self-incompatibility (ISI) for *Vatica yeechongii* is 0. This can concluded that *V. yeechongii* is completely self-incompatible.

Figure 4.23: Fruit occurred through cross-pollination treatment

Table 4.9: Results of pollination experiments (compatibility studies) in *V. yeechongii*

Test	Treatment	No. of flowers pollinated	No. of flowers set fruit	Fruit set (%)
Open pollination	Inflorescences tagged without manipulations	60	17	28.3
Control for selfing	Flowers bagged with no manipulations	60	0	0
Self-pollination (Geitonomy)	Flowers emasculated, bagged and pollen from other flowers of the same tree applied	60	0	0
Cross-pollination (Xenogamy)	Flowers emasculated, bagged and pollen from another tree applied	60	8	13.3
Emasculation (Agamospermy)	Flowers emasculated and bagged	60	0	0

4.4.2 Potential pollinators

Observation for the potential pollinators were made starting from 0600-1200 hours for five days. Detailed observations were made on 17 March 2014 by counting the frequency of occurrence of the insects for five minutes at 30 minutes interval (Table 4.10). The list of insects observed on flowering trees of *Vatica yeechongii* is presented in Table 4.11. Four orders of insects has been identified as visitors of *Vatica yeechongii,* Hymenoptera, Thysanoptera, Diptera and Coleoptera. The most frequent visitors were the bees and thrips which belong to the orders Hymenoptera and Thysanoptera. They were seen almost throughout the study period. Other insect visitors such as ants, flies and small beetles were also observed and collected.

Generally, the insects started their activities and visited the flower early morning around 0630 hours, when the petals started to open. The number of insects increased around 0700-0800 hours, coinciding with the time of stigma becoming receptive. No insects were observed after the petals and anthers started to fall around 0930 until 1130 hours hours except for the ants that were crawling and moving around on the inflorescences and the branch of *Vatica yeechongii.*

The biggest number of insects observed on the open flowers is from the family Thripidae (Figure 4.24). They were found crawling on the petals and some of the thrips were moving deep inside the open flower. It is hard to monitor the movement of the thrips as they were too small and they easily escape (Figure 4.25). Despite the dominance, not all Thripidae carried the pollen on their bodies. Only two *Thrips* sp. was found to carry the pollen on their abdomens (Figure 4.26).

The bees belonging to the subfamily Apinae were seen to visit the flower and inflorescences within and inter trees (Figure 4.27). They come early in the morning and tried to get in even the flowers not were fully open. They remained on the opened flower for about 5-30 seconds, sometimes until 60 seconds. They crawled and tried to get deep into the flower

by inserting their heads and abdomens in order to reach the pollen located around the base of floral bracts. They would move to another open flower either on the same or different inflorescences within and inter tree. While foraging on other flowers they would subsequently transfer the pollen onto the stigmas. From observation using scanning electron microscope (SEM), all 12 specimens of *Geniotrigona thoracia, Heterotrigona itama* and *Tetragonula laeviceps* carried a large amount of pollen on their bodies; mouth part, legs and abdomen (Figure 4.28, Figure 4.29).

The ants identified as *Dolichoderus thoracicus* and *Pseudolasius* sp. from the family Formicidae were also seen crawling on the trees including on the trunk, branch, inflorescences and also open flowers, but rarely seen moving inside the flowers (Figure 4.30). They have nesting on the branch of the trees. Based on the observations, these ants search for food aimlessly and pollen grains were not their main target of food. Besides, only one specimen of *Psedolasius* sp. had few pollen loads on tip of abdomen (Figure 4.31).

Other insects seen and caught during the observation were flies, *Bactrocera* sp. under the family Tephritidae might be attracted by the sweet scented opened flowers (Figure 4.32). However, the numbers of flies visiting the flowers and inflorescences were very low compared to the Apidae.

The leaf beetle from the family Chrysomelidae also visited the opened flowers of *V. yeechongii*. Two beetle species have been observed. The small beetles (Figure 4.33) fed on and destroyed petals of the flowers. Some individuals crawled on the flower and sometimes moving inside the flower and perhaps ate the pollens. This foraging behaviour may lead to contact with an active stigma. The larger beetle species (Figure 4.34) ate the petals only and did not probe inside the flowers where the anthers are located. The size of the beetle is large hence did not fit the corolla tube. These leaf beetles also carried little pollen on their abdomens.

Table 4.10: Frequency of insect visitation on opened flowers, from 0530-1200 hours, 17 March 2014

	Frequency of visit												
Hour / Family	**0530 – 0600**	**0600 – 0630**	**0630 – 0700**	**0700 – 0730**	**0730 – 0800**	**0800 – 0830**	**0830 – 0900**	**0900 – 0930**	**0930 – 1000**	**1000 – 1030**	**1030 – 1100**	**1100 – 1130**	**1130 – 1200**
Formicidae	-	-	-	1	1	3	-	1	2	1	-	-	-
Apidae	-	-	1	1	3	5	8	7	4	2	1	1	-
Thripidae	-	2	5	5	7	10	11	13	9	4	3	-	-
Tephritidae	-	-	-	-	-	-	1	-	1	1	-	-	-
Chrysomelidae	-	-	-	1	1	2	3	2	2	1	-	-	-

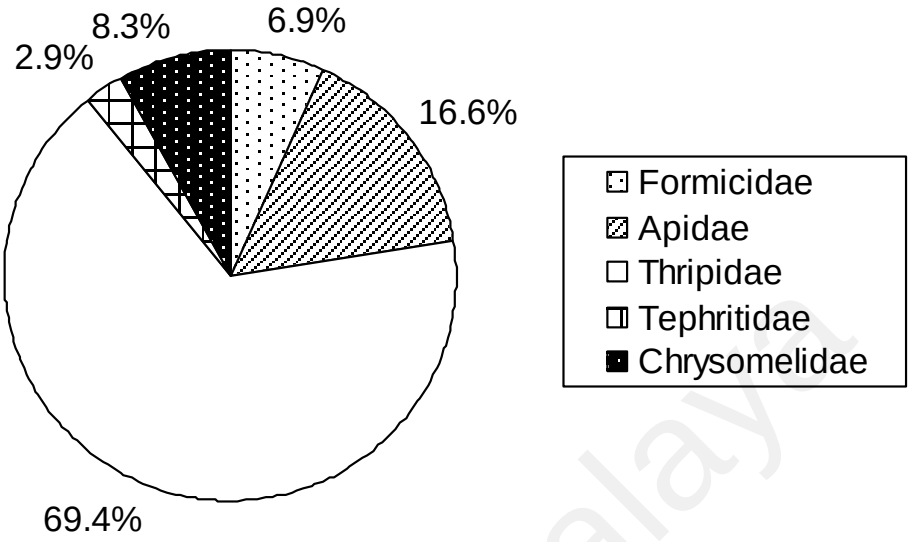

Figure 4.24: Percentage of floral visitors based on family

Figure 4.25: *Thrips* sp. from family Thripidae

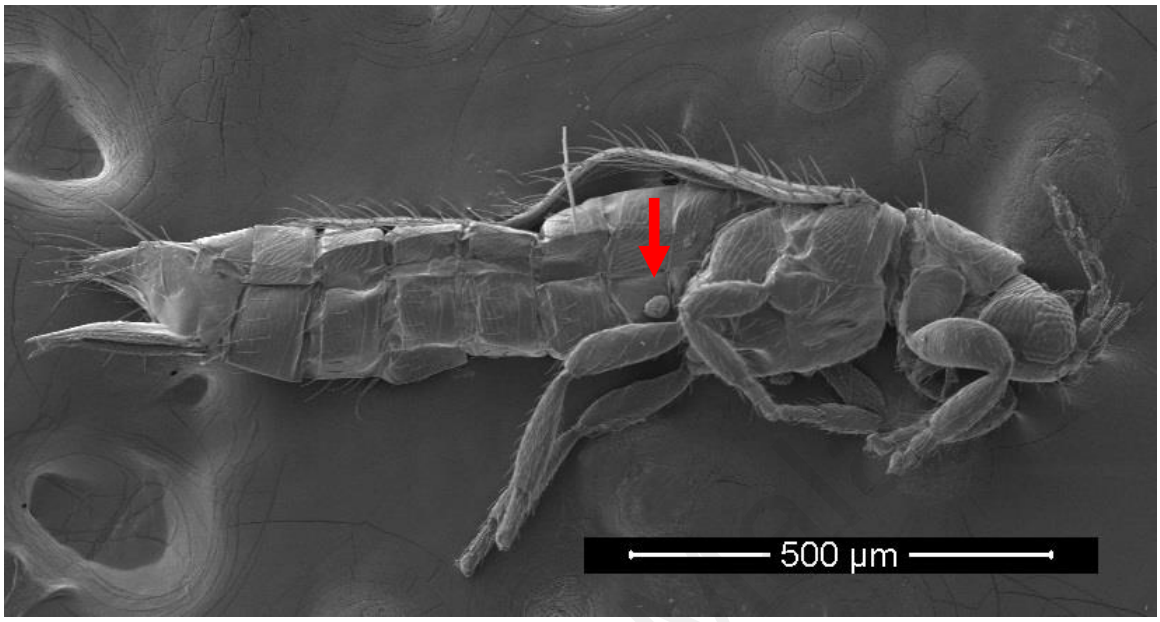

Figure 4.26: Pollen grains at abdomen of *Thrips* sp.

Figure 4.27: *Geniotrigona thoracia* from subfamily Apinae

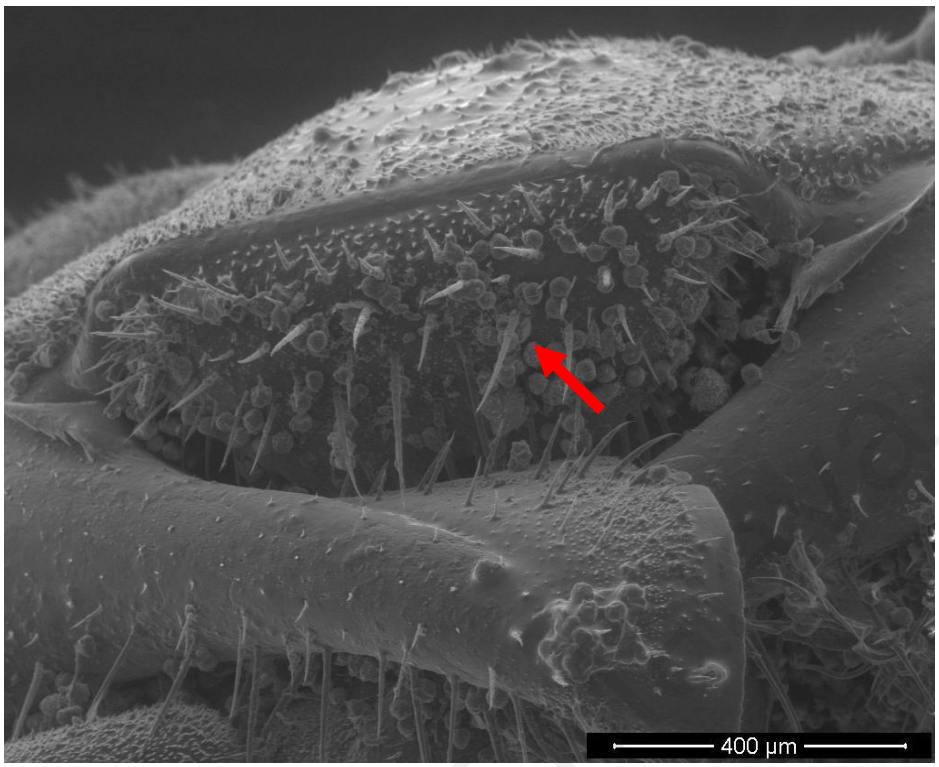

Figure 4.28: Pollen grains at mouth part of *Geniotrigona thoracia*

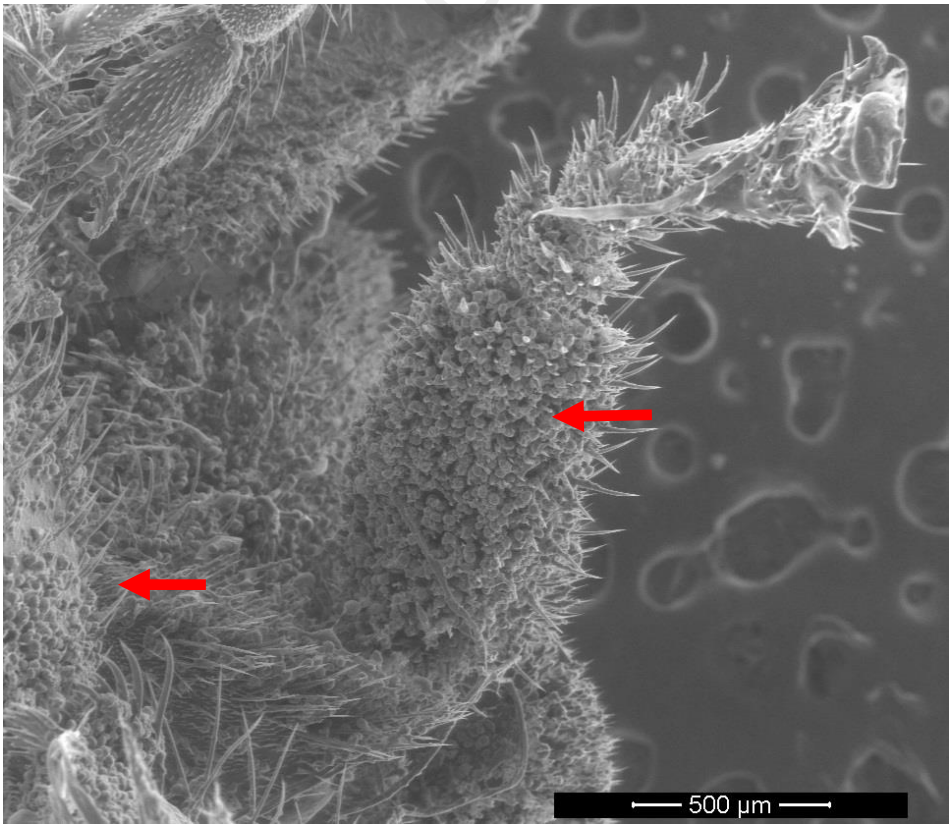

Figure 4.29: Pollen grains at legs and abdomen of *Geniotrigona thoracia*

Figure 4.30: *Dolichoderus thoracicus* from family Formicidae

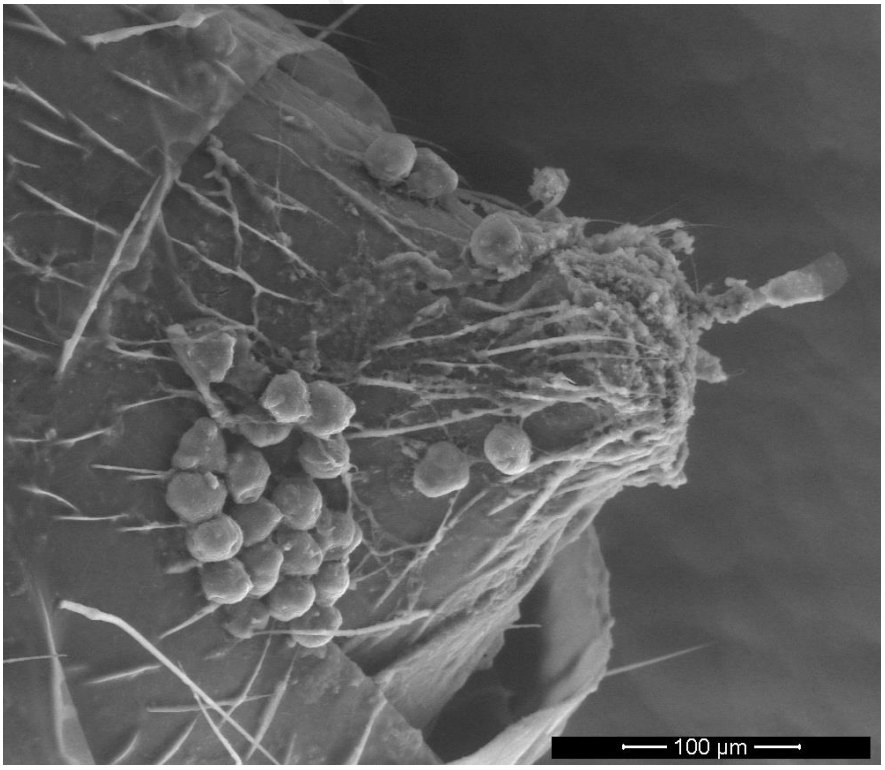

Figure 4.31: Pollen grains at tip of abdomen of *Pseudolasius* sp.

Figure 4.32: *Bactrocera* sp. from family Tephritidae

Figure 4.33: Small beetles from family Chrysomelidae

Figure 4.34: Large beetles from family Chyrsomelidae

Table 4.11: Insects identified from the flowers of *Vatica yeechongii*

No.	Order	Family (Subfamily)	Species
1.	Hymenoptera	Formicidae (Formicinae)	*Dolichoderus thoracicus* (Figure 4.35) *Pseudolasius* sp. (Figure 4.36)
		Apidae (Apinae)	*Geniotrigona thoracia* (Figure 4.37) *Heterotrigona itama* (Figure 4.38) *Tetragonula laeviceps*
2.	Thysanoptera	Thripidae (Thripinae)	*Thrips florum* (Figure 4.39) *Thrips hawaiensis* (Figure 4.40) *Thrips palmi* *Thrips* sp. (Figure 4.41) *Lefroyothrips* sp.
3.	Diptera	Tephritidae (Dacinae)	*Bactrocera* sp. (Figure 4.42)
4.	Coleoptera	Chrysomelidae	Unidentified small beetle (Figure 4.43), Unidentified large beetle (Figure 4.44)

Figure 4.35: *Dolichoderus thoracicus*

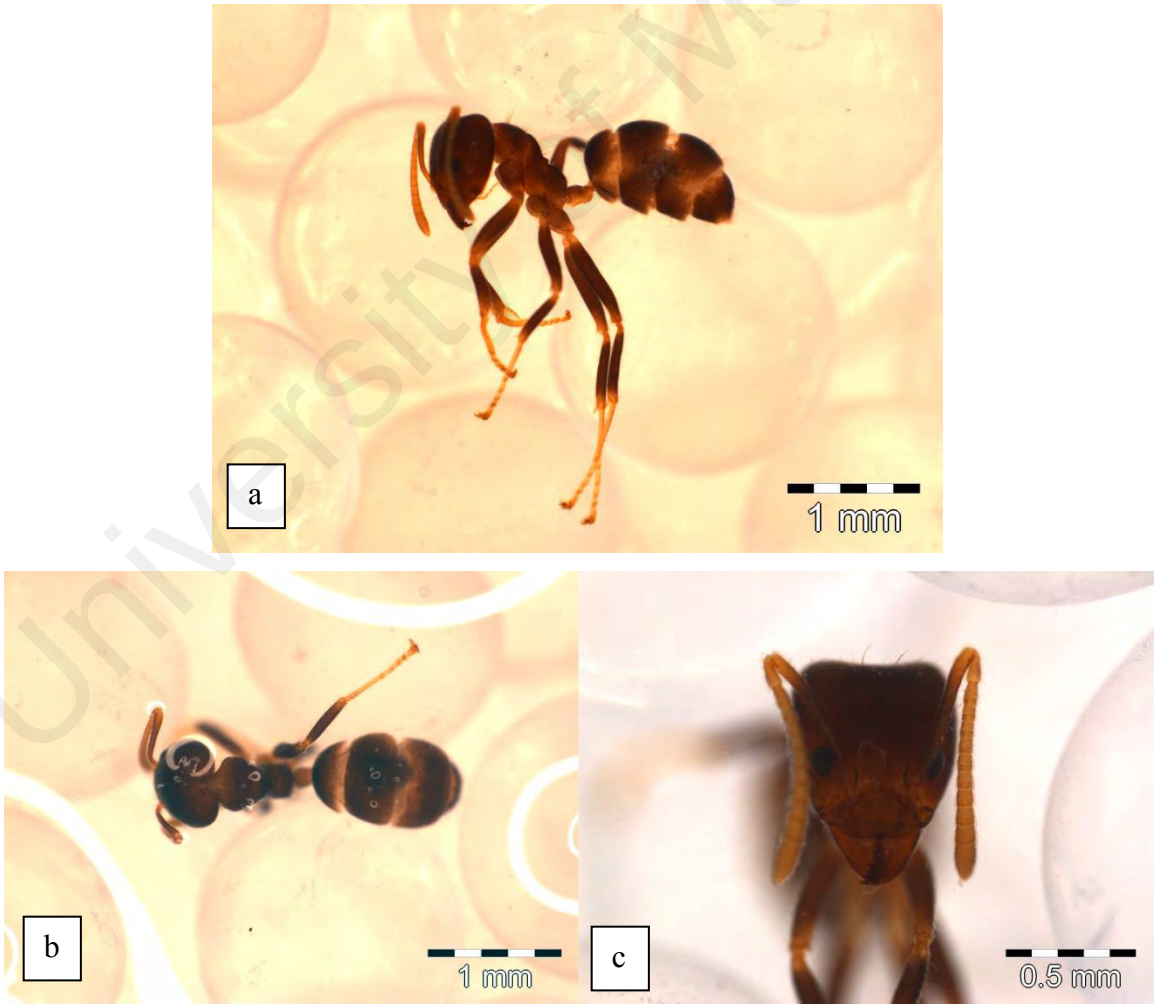

Figure 4.36: *Pseudolasius* sp. (a) Lateral view. (b) Dorsal view. (c) Head and mouthpart.

Figure 4.37: *Geniotrigona thoracia*

Figure 4.38: *Heterotrigona itama*

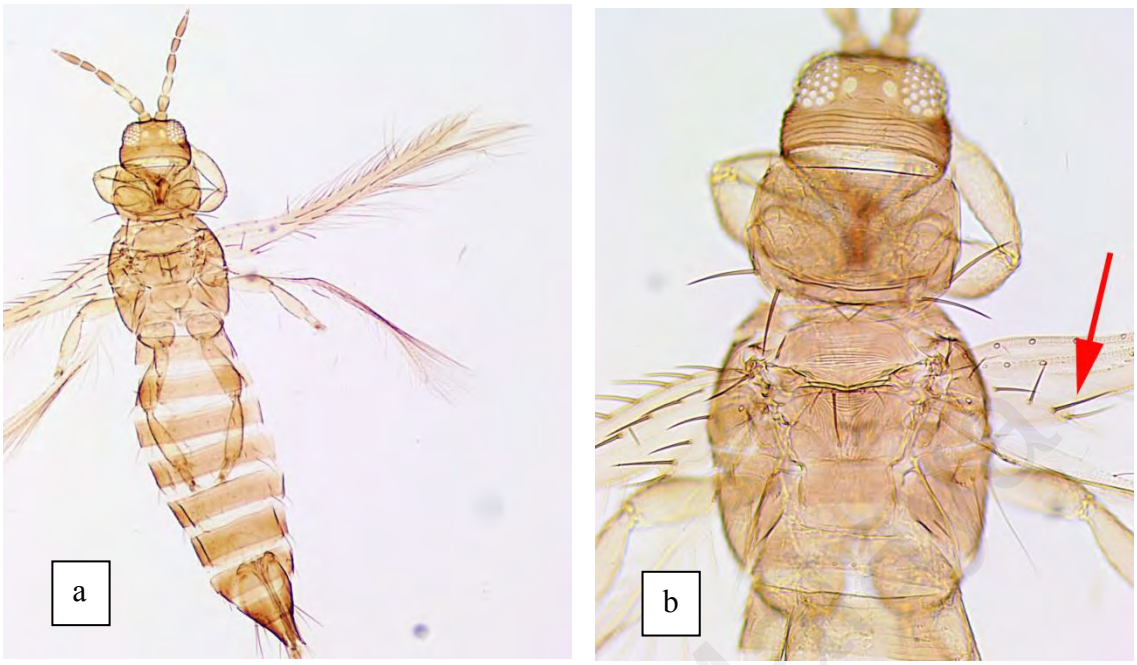

Figure 4.39: *Thrips florum* (a) Dorsal view. (b) Head

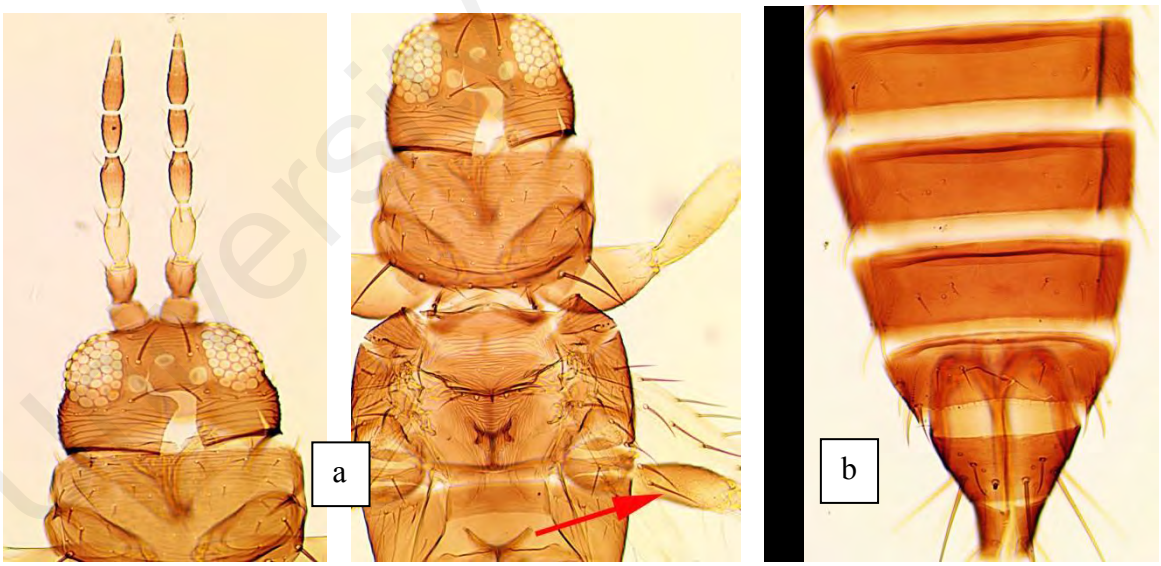

Figure 4.40: *Thrips hawaiensis* (a) Head. (b) Abdomen.

Figure 4.41: *Thrips* sp. (a) Lateral view. (b) Dorsal view

Figure 4.42: *Bactrocera* sp.

Figure 4.43: Small beetle from the family Chrysomelidae

Figure 4.44: Large beetle from the family Chrysomelidae

4.4.3 Pollen to ovule ratio

The flower of *Vatica yeechongii* has fifteen anthers per flower and mean pollen grains per anther was 1036.4, SD 93.33 (Figure 4.45). One flower was estimated to have a total of 15,546 pollen grains. Total number of ovules per flower was 6 and the ratio of pollen grains to ovules was 2591:1. According to Cruden (1977), the breeding system for *Vatica yeechongii* was obligate outcrossing or xenogamy.

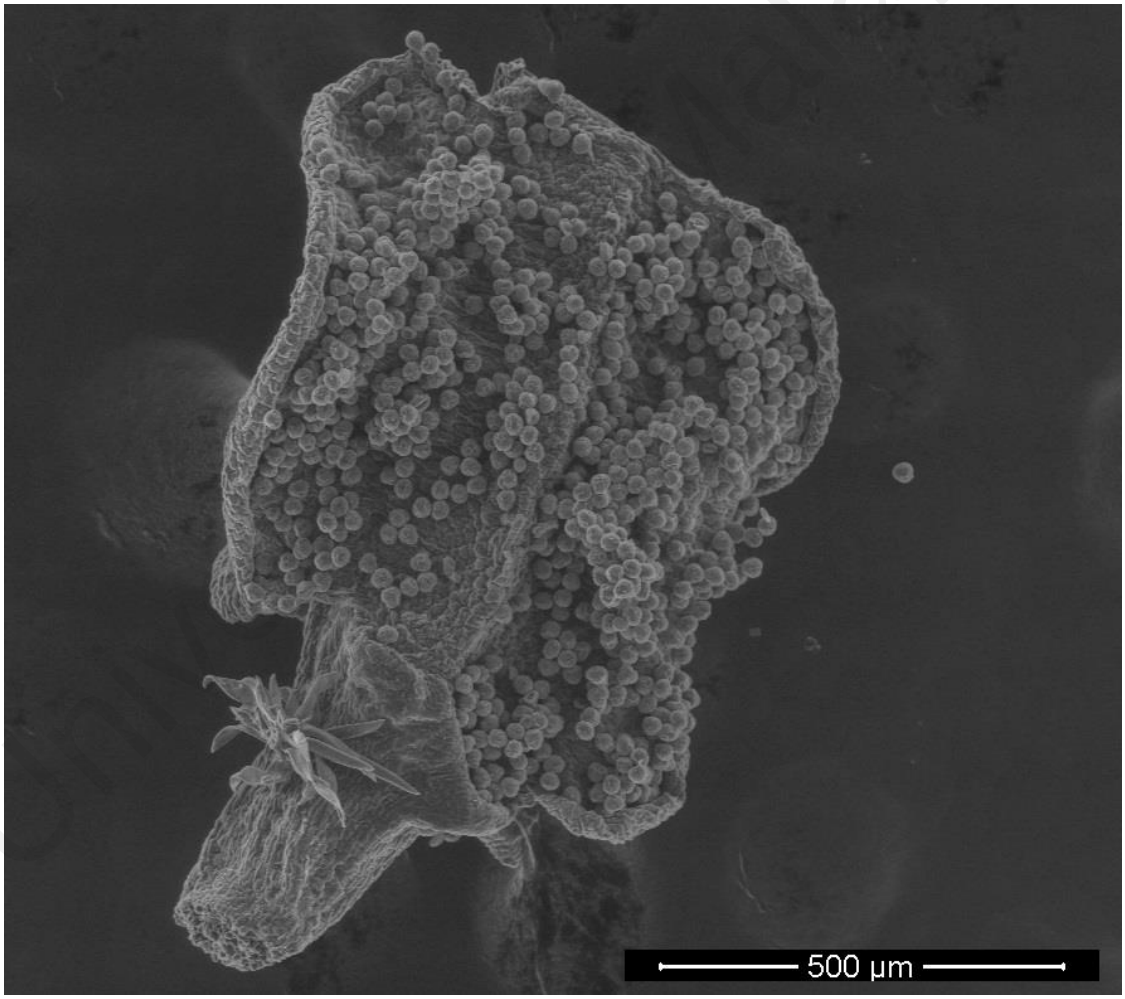

Figure 4.45: SEM showing pollen grains per anther

4.5 Pollen

4.5.1 Morphology

The pollen grains of *Vatica yeechongii* was oblate and tricolpate (Figure 4.46). The apertures was shorter than length of the grain at equatorial view. The outer layer of the exine was coarsely reticulate (Figure 4.47). According to Erdtman (1969), the pollen grains were categorized as medium-sized (25.0 – 50.0 µm). The polar-axis (Figure 4.48) for untreated pollens ranges from 24.19 µm to 27.76 µm, while the equatorial-axis (Figure 4.49) ranges from 19.85 µm to 24.96 µm (see Table 4.12). It is visible to the naked eyes as white dust. For acetolysed pollens, it has bigger size as polar-axis ranges from 28.39 µm to 38.76 µm, while equatorial-axis ranges from 21.08 µm to 27.65 µm. The P/E ratio (in percentage) was 136 and 116, for the acetolysed and untreated pollen grains respectively. The shape was subprolate for the untreated pollen grains, and prolate for the acetolysed pollen grains, according to Erdtman (1969).

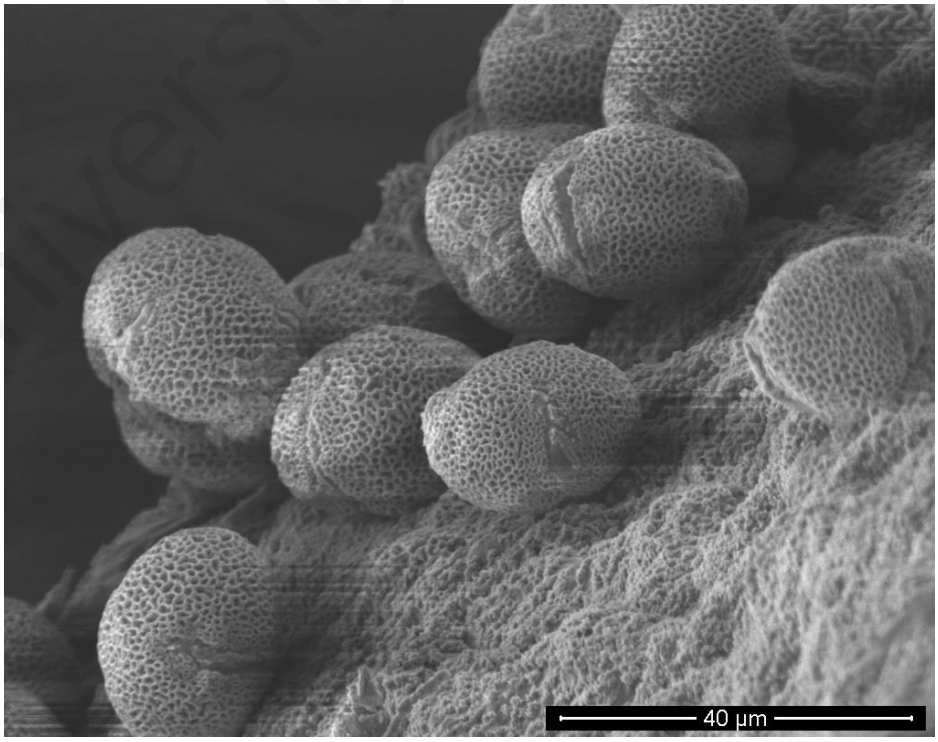

Figure 4.46: SEM showing pollen grains of *Vatica yeechongii*

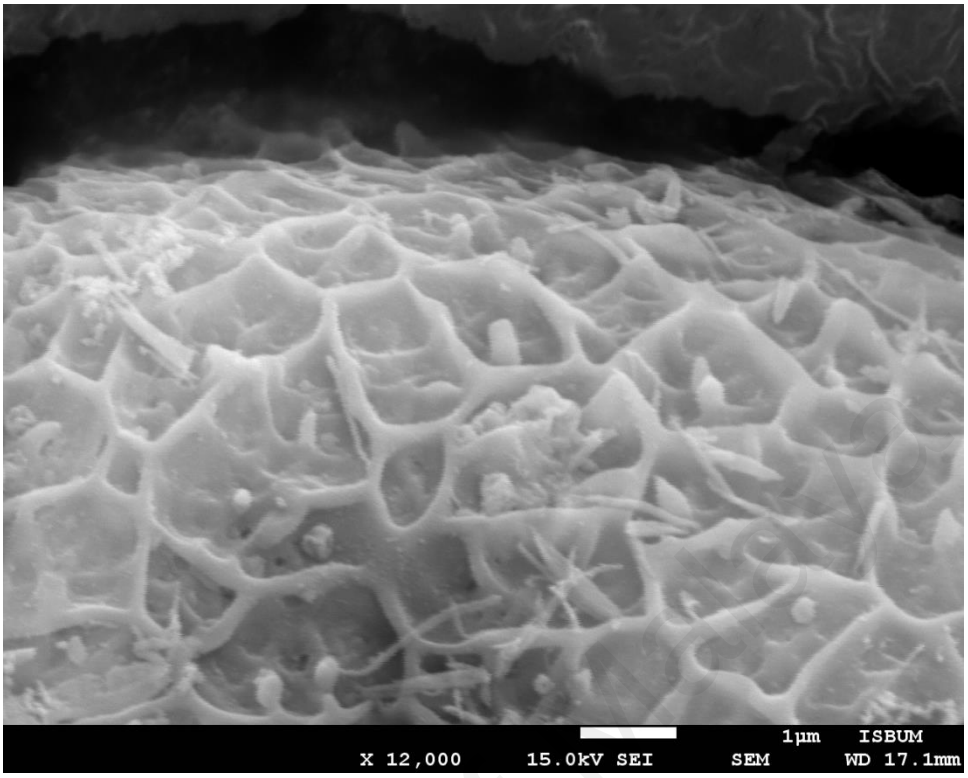

Figure 4.47: SEM showing the exine of the pollen was coarsely reticulate

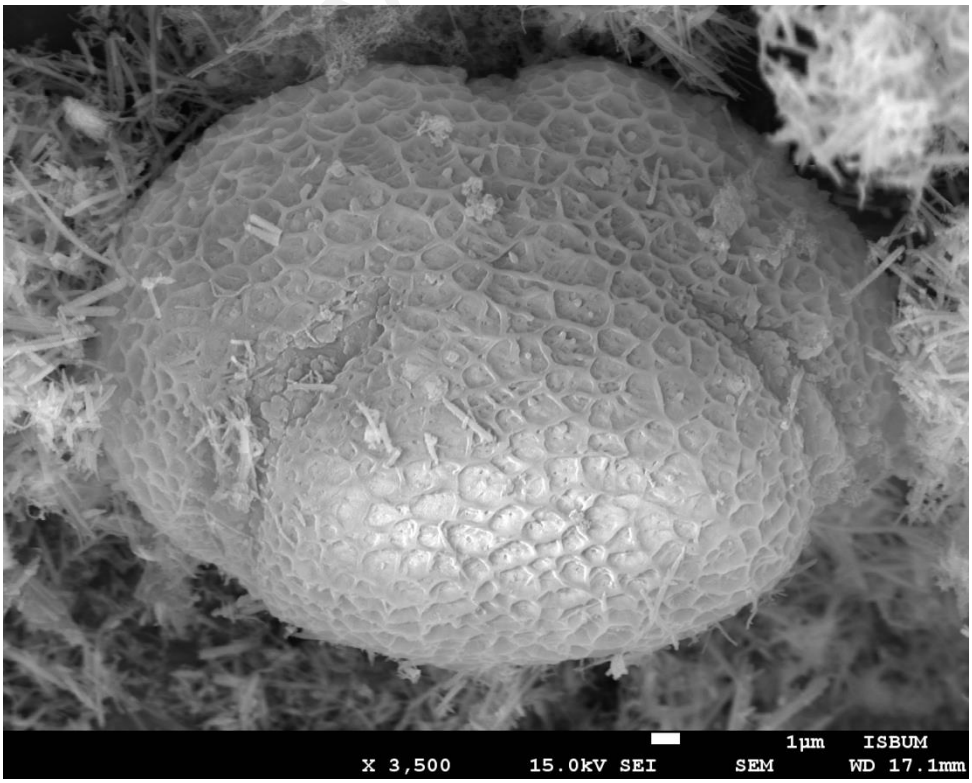

Figure 4.48: SEM showing the polar view of the pollen

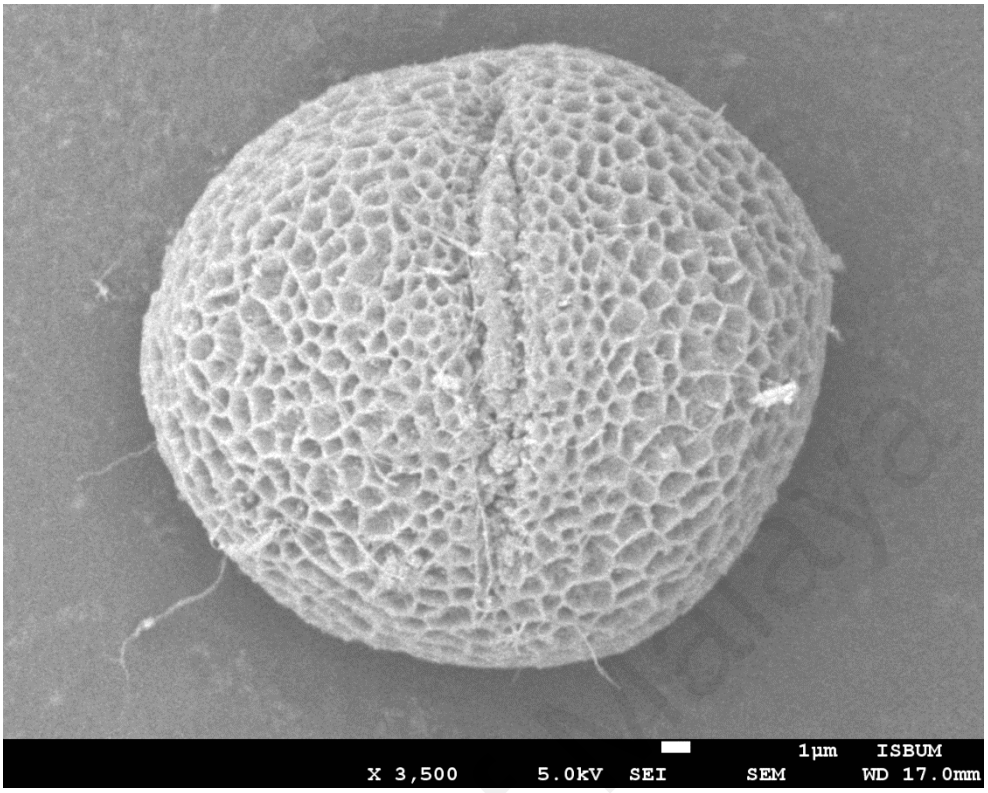

Figure 4.49: SEM showing the equatorial view of the pollen

Table 4.12: Measurements of acetolysed and untreated pollen grains; P – polar length, E – equatorial length

	Acetolysed		**Untreated**	
	P	**E**	**P**	**E**
N	35	30	22	22
Mean length (µm)	32.87	24.16	26.02	22.35
SD	2.37	1.74	1.18	1.26
Median length (µm)	33.15	23.93	26.30	22.47
Min length (µm)	28.39	21.08	24.19	19.85
Max length (µm)	38.76	27.65	27.76	24.96
P/E X 100		136		116

4.5.2 Germination and viability

The importance of this experiment is to study the ability of pollen to germinate. From the preliminary experiments done, the pollen of *V. yeechongii* did not show any sign of germination in distilled water with 0.01% boric acid and 5% sucrose solutions without boric acid. In media 10% and 15% sucrose, each without boric acid, the pollen seemed viable as there are signs of pollen tubes emerging. However, it is not considered as germination if the length of its tube is less than the diameter of the pollen grains. Meanwhile, the pollen germinated in media of 5%, 10% and 15% sucrose solutions with 0.01% boric acid (b.a). Even though the pollen is tricolpate, it produces only one pollen tube (Figure 4.50, Figure 4.51).

The percentage of pollen germination in media of 5%, 10% and 15% sucrose solutions with boric acid are 3.43%, 3.95% and 7.59% respectively (Table 4.13). Therefore, 10% and 15% sucrose solutions with 0.01% boric acid were chosen as media for subsequent replicates as both showed higher percentage of germination compared to media of 5% sucrose solutions with 0.01% boric acid. Pollen grains of *Vateria macrocarpa* (Dipterocarpaceae) had germinated well in 15% sucrose concentration. Pollen viability of *V. macrocarpa* was seen up to two days in germination media (Keshavanarayan *et al*., 2015).

Table 4.13: Percentage of pollen germination in media of 5%, 10% and 15% sucrose solutions with boric acid

	5% sucrose solutions + 0.01% boric acid	**10% sucrose solutions + 0.01% boric acid**	**15% sucrose solutions + 0.01% boric acid**
Total pollen	262	152	158
Pollen germinated	9	6	12
Not germinated	253	146	146
% germinated	3.43	3.95	7.59

Pollen grains of *V. yeechongii* showed germination after one and half hour incubated in the sucrose. Pollen age 4 hours after anthesis had the highest rate of germination 38.80% which were incubated in 15% sucrose + 0.01% boric acid, followed by the pollen age 2 hours 28.57% and the pollen age 8 hours 27.64% (both also incubated in 15% sucrose + 0.01% boric acid) (Figure 4.52). As for 10% sucrose + 0.01% boric acid, the highest germination rate is pollen age 4 hours 24.01% and followed by the pollen age 8 hours 23.48%. The lowest germination rate is pollen aged 2 hours after anthesis that incubated in 10% sucrose + 0.01% boric acid (8.86%).

Meanwhile, the highest mean tube length is the pollen age 2 hours incubated in the medium of 15% sucrose + 0.01% boric acid (497.08 µm, SD 205.36), followed by the pollen age 4 hours incubated in 10% sucrose + 0.01% boric acid (466.37 µm, SD 201.53) and the pollen aged 2 hours (456.29 µm, SD 367.27) incubated in 10% sucrose + 0.01% boric acid. The lowest mean tube length is the pollen aged 42 hours after anthesis incubated in 15% sucrose + 0.01% boric acid (61.95 µm, SD 11.11). The maximum tube length recorded was 1096.64 µm, from pollen aged 2 hours incubated in the medium of 10% sucrose + 0.01% b.a.

Figure 4.53 showed that pollen grains of *V. yeechongii* performed and germinated well in the medium of 15% sucrose + 0.01% boric acid compared to the medium of 10%

sucrose + 0.01% boric acid. Pollen aged 4 hours after anthesis showed healthy germination in both media. Besides, the length of pollen tube reduced rapidly after 4 hours of pollen anthesis and the pollen grains are still viable even at 42 hours after anthesis. The pollen grains need sucrose in the media for germination as the addition of boric acid in distilled water did not show any sign of pollen germination. The addition of 0.01% boric acid in sucrose solution seemed to increase the pollen germination and the length of pollen tubes.

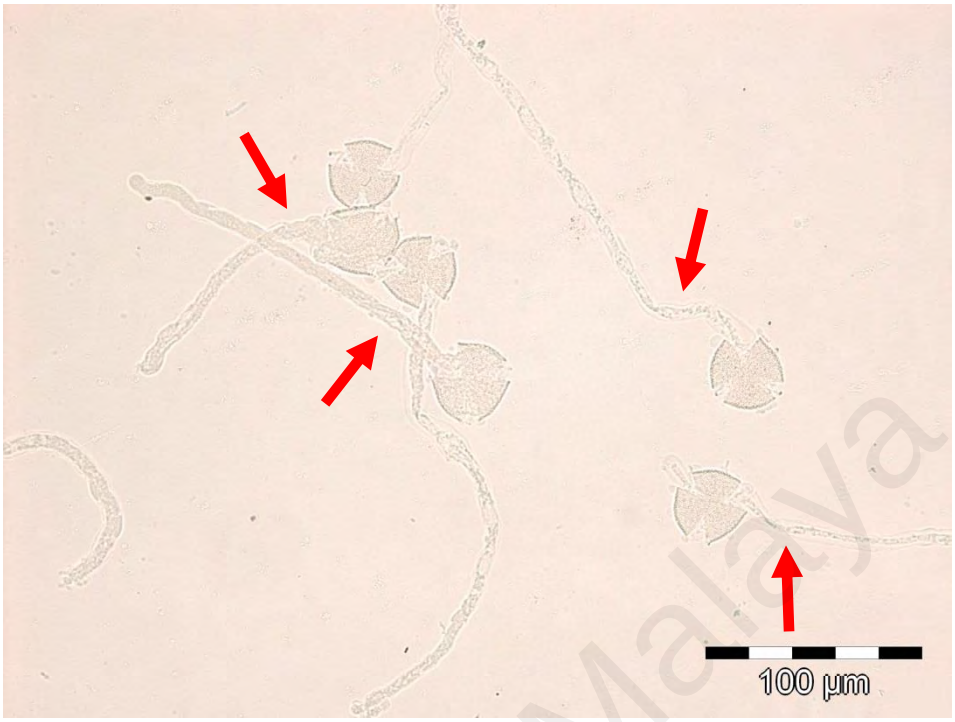

Figure 4.50: Pollen germination with one pollen tube

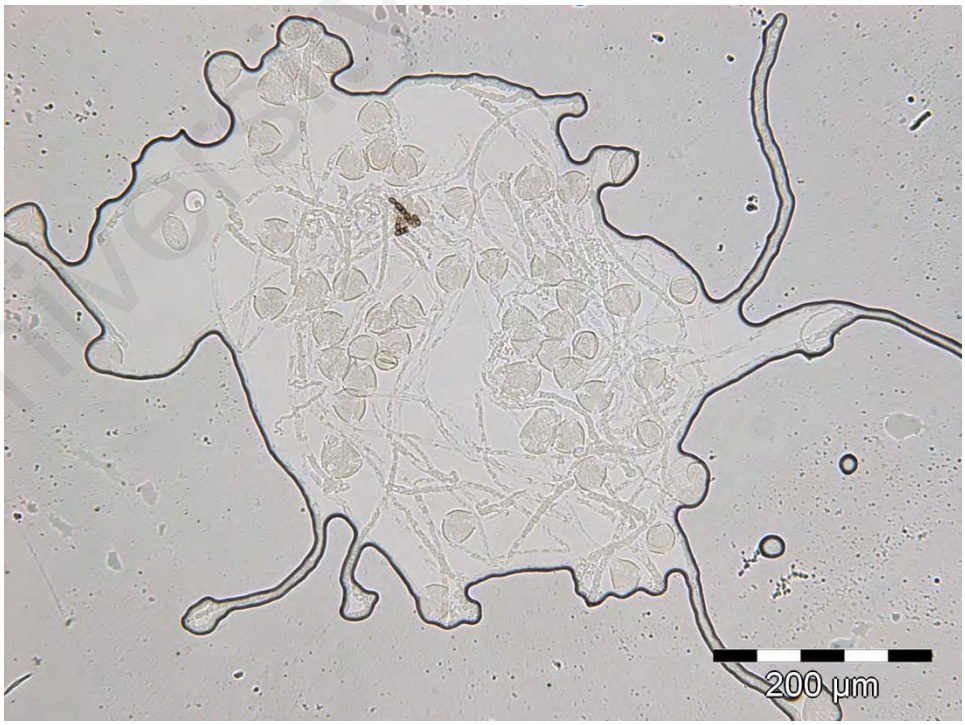

Figure 4.51: Pollen germinated in media 15% sucrose solution with 0.01% boric acid added

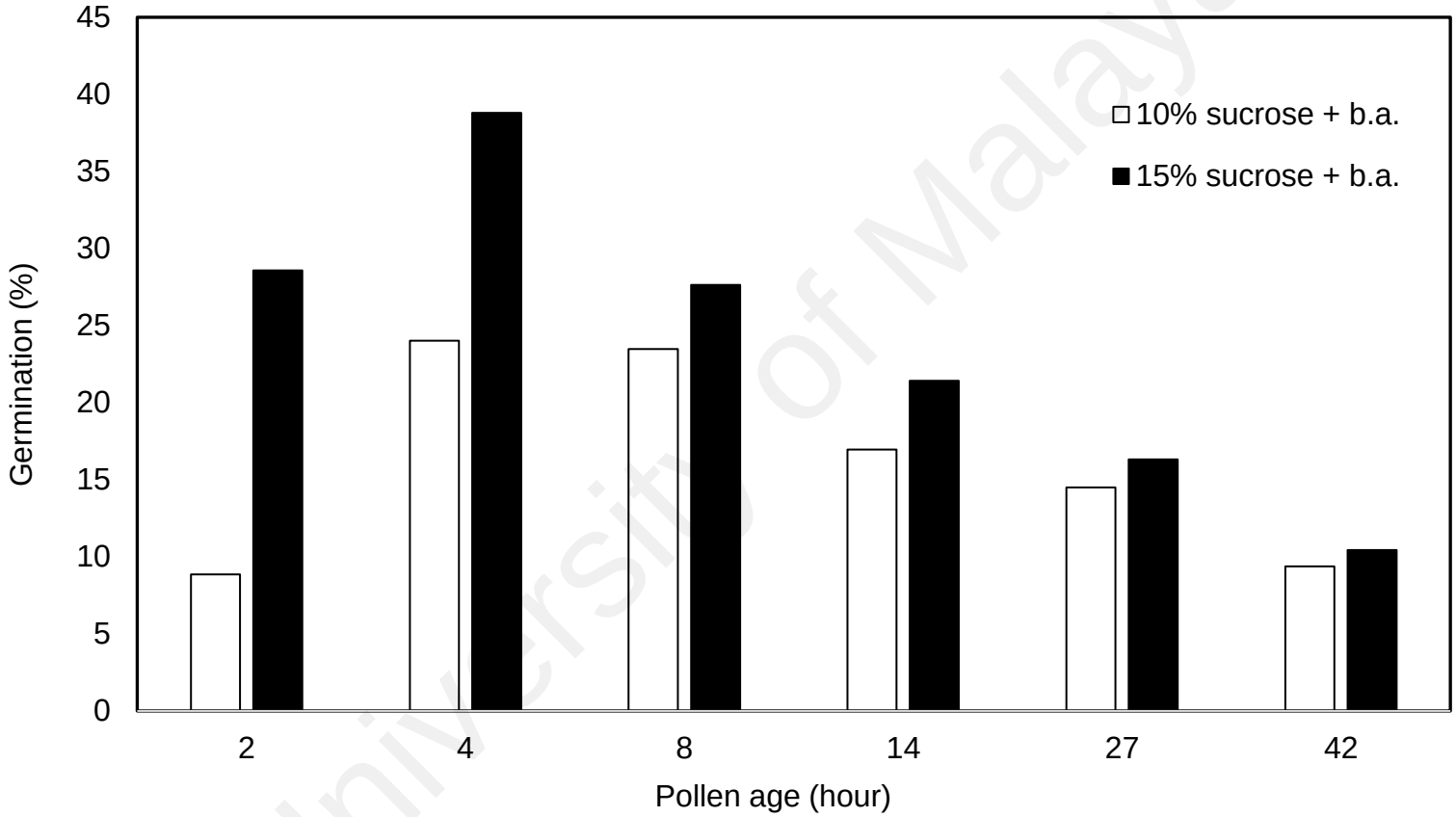

Figure 4.52: Percentage germination of pollen at different age after anthesis

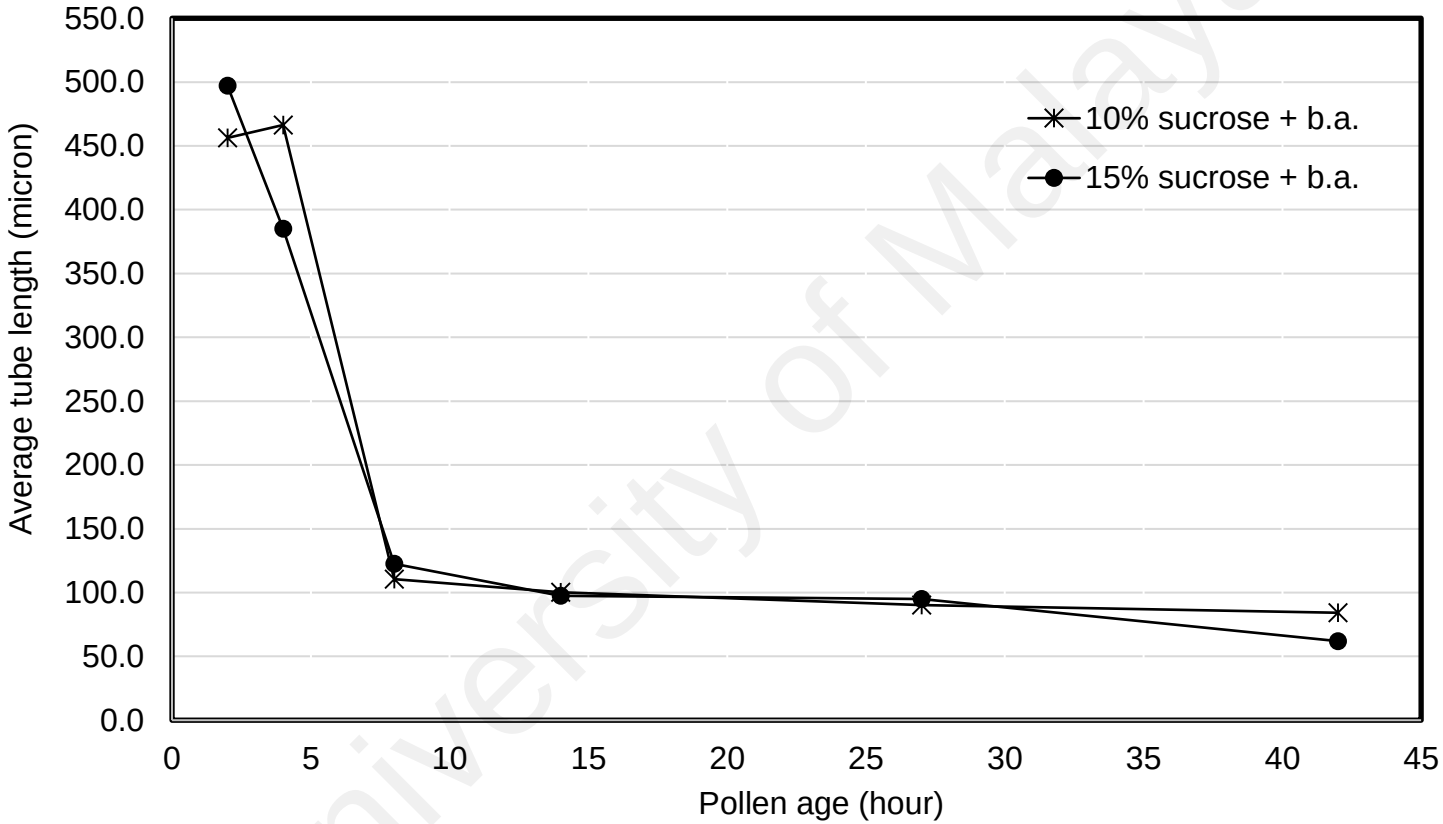

Figure 4.53: Pollen tube length in different media

4.6 Seed biology

4.6.1 Seed Germination

Three types of media were used for sowing; 100% soil, 100% sand and sandy soil mixed with the ratio 2:1. The fresh seeds germinated rapidly and showed high percentage of germination in sandy soil as media for sowing. The highest percentage was 97.5% which is sand as media for sowing, followed by sand 95% and soil 77.5% (Table 4.14).

The best performance was showed by seed germinated in sand as it took two weeks to fully germinate compared to soil and sandy soil which both took about 5 weeks. Seed germination type of *V. yeechongii* is epigeal. The radicle emerges through the seed coat and heading into the sowing media (Figure 4.54), while the hypocotyl elongates together with cotyledons. The colour of the cotyledons after emerges from the seed coat are reddish and then changed to green (Figure 4.55).

The first pair of leaves is opposite and seed using soil as media for sowing showed the first appearance of paired leaves (Figure 4.56). The leaves are formed 15 days after the cotyledons expand. Followed by sand, where the leaves are formed after 16 days and 18 days for seed using sandy soil as media for sowing. The next leaf to appear is single and alternate (Figure 4.57).

Table 4.14: Percentage of germination of *V. yeechongii* (sowing date 20th August 2014)

Germination	Media for sowing		
	Soil	**Sand**	**Sandy soil**
Total number of seeds	40	40	40
Number of germinated seeds	31	38	39
Number of ungerminated seeds	9	2	1
Percentage seed germination (%)	77.5	95	97.5
Average length of cotyledon (cm)	5.7	5.6	5.9
Germination period (weeks)	5	2	5
Appearance of first pair of leaves (number of days after sowing)	15	16	18
Type of germination	Epigeal	Epigeal	Epigeal

Figure 4.54: The radicle emerges through the seed coat

Figure 4.55: The colour of the cotyledons changed from reddish to green

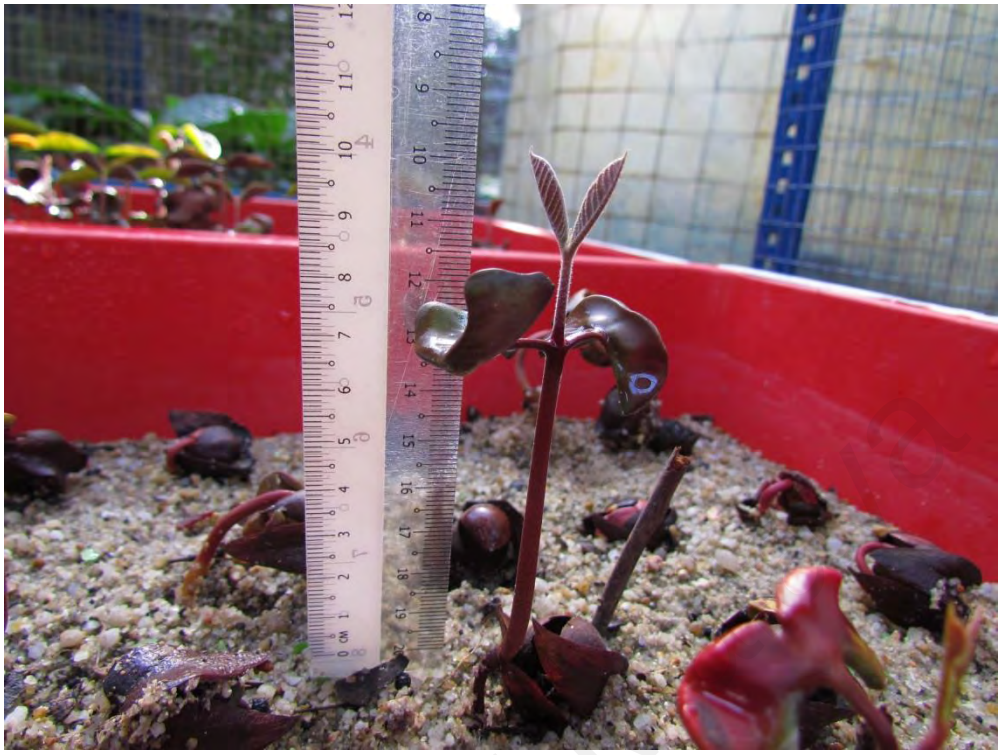

Figure 4.56: The first pair of leaves emerges through the seed coat is opposite

Figure 4.57: The next leaf to appear is single and alternate

CHAPTER 5: DISCUSSION

5.1 Morphology

Vatica yeechongii is an entomophilous species, where the anther dehisce and stigma receiptivity are asynchronous. Flowers start to open at early dawn around 0530 hours and at the same time anther dehisce the pollen, followed by stigma receptivity 30 minutes later. *V. yeechongii* had many flowers per inflorescence. The funnel-shaped flowers with creamy white colour gives a contrast tone with the green colour of the leaves. In addition, the flowers of *V. yeechongii* produced a very strong sweet scent which enhances their ability to attract pollinators.

The relations among pollination systems and multiple floral characters such as colour, shape and odour known as pollination syndromes are considered as secondary attractants, played important roles in attracting potential pollinators (Faegri & Van Der Pijl, 1979). Cup-shaped and rotate flowers were common in plants pollinated by social bees, beetles and diverse insects (Momose *et al*., 1998).

5.2 Flower and fruit development

The growth of flower bud from the inflorescence initiation required between 26 to 32 days. By the third week after a successful pollination, young fruits begin to develop. The growing process of young fruits into falling of matured fruits takes about 80 to 90 days. Most young fruits abort at early stage of fruits development. Lack of nutrient possibly caused abortion among young fruits. It has also been reported that *Shorea* species (section Muticae) had extremely high number of immature fruits in abortion during the first 2 weeks after the flowers bloom (Chan, 1977).

Usually only 1-5 fruits per infructescence managed to reach matured stage. Some of the fruits did not reach the matured stage as they were eaten by animals such as monkeys and squirrels. This scenario happens during fruiting period in 2012 and 2013 where not many trees in that area flowering and fruiting. Thus, the animals tend to choose fruits of *V. yeechongii* as their source of food. In 2014, where many trees surrounding the area were in mast fruiting, the monkeys did not consume *Vatica* fruits.

5.3 Phenology

From the observations made on the flowering phenology, it can be concluded that *V. yeechongii* flower had annual flowering with the capability to produce flowers twice a year. First flowering period occurs in February to April, while next flowering occurs in September to November. However, not all trees had the capability to produce flowers twice a year. There is different intensities of flower between these two flowering periods. More trees are flowering and the intensity of flowers per tree is higher in the first cycle and trees fruited profusely compared with the second cycle of flowering where the number of flowering trees and flowering intensity is low resulted in little or no fruit set.

Sporadic flowering occurred in February 2012 and March 2013 as only few numbers of trees produced flower. Gregarious flowering took place in February 2014, when all seven trees at Sungai Lalang FR and ten out twenty five trees at Setul FR developed flowers simultaneously and this was followed by profuse fruiting. Besides, other Dipterocarpaceae species near the population at Sungai Lalang FR such as *Shorea leprosula, S. bracteolata* and *S. parvifolia* were also flowering with high intensity. Non-Dipterocarpaceae species such as *Saraca cauliflora* (Leguminoseae), *Parkia* sp. (Leguminosae), *Canarium* sp. (Burseraceae) and other trees were also flowering. These was supported by previous study on *Shorea* species (section Muticae) in Pasoh FR by Chan (1977) who observed species from

other genera of Dipterocarpaceae namely, *Dipterocarpus*, *Vatica*, and *Hopea* were also mast fruiting alongside with certain species from family Bombacaceae, Leguminosae, Burseraceae and Euphorbiaceae. Lau (2015) mentioned that general flowering occurred in many tree families in several forest reserves in Terengganu, Johor, Selangor, Pulau Pinang and Perak during that dry season which lasted from February to April. He also stated that trees of *Saraca thaipingensis*, *Mesua* sp., *Diospyros* sp., *Syzygium* sp., *Shorea* sp. and *Dipterocarpus* sp. were flowering during his phenological observation on *Aquilaria malaccensis* which is flower heavily in Pulau Pinang and Perak.

General flowering is an event when most dipterocarps and many other trees flower and fruit profusely (Ashton *et al*., 1988; Appanah, 1993; Sakai, 2002). *V. yeechongii* flowering with high intensity was in synchrony to the general flowering, as shown in the annual flowering trees species where the trees fruited profusely in a general flowering year (Sakai, 2002).

Based on monthly rainfall from year 2012 until 2014, there are notable differences in the amount of total rainfall at early year of 2014. Both weather stations had a low rainfall in the first three months compared with year 2012 and 2013. Coincidently, dry spell occurred in many parts of the country starting early February 2014, which lasted for about three months. Sakai *et al.* (2006) reported that drought trigger species masting in the aseasonal tropical forests, and it coincided with masting flowered of *V. yeechongii* occurred in 2014.

There are possibilities that a drop in daily minimum temperature trigger the initiation of inflorescences of *V. yeechongii* at Sungai Lalang FR and Setul FR, hence inducing high intensity flowering. Ashton *et al.* (1988) concluded that a drop in daily minimum temperature is the most likely meteorological signal that triggers the general flowering. This conclusion was based on correlation between general flowering and El Niño phenomenon. A drop in

minimum temperature up to 3ºC were recorded 1-2 months befores general flowering began in a lowland dipterocarp forest of Sarawak (Sakai *et al*., 1999).

Based on statistical analysis done, there is no significant correlation between the number of flowering trees with meteorological factors such as rainfall and minimum temperature. It might be due to the small population sizes and inadequate number of samples from both populations, thus difficult to observe the correlation between the factors mentioned above. *V. yeechongii* flowered annually but masting in 2014 coincided with drought and drop in mean minimum temperature.

V. yeechongii flowers every year and had interval or resting periods for 1-2 months after fruiting ends in the first period. Usually, only few number of trees produced flower and intensity of flowering is low when the trees started to produce flower in the second periods. This may be due to the trees had limited resources after exhaustive synchronous flowering in the first period or no signal from meteorological factors to induce or push the trees to produce more flowers.

Flowering pattern of *V. yeechongii* can be predicted at the population level as both populations flower about the same time. The ability of trees at Sungai Lalang FR to flower twice a year compared with Setul that flowered once a year indicates that the different factors affect the individual trees to produced flower either biotic (e.g. genetics, plant physiology, pollinators) or abiotic (e.g. niche, weather, water, soil, nutrient availability).

Based on the estimation of flower production, there are great different in the number of flowers for each trees. Age of the trees is the most likely the factor that contributed to this situation as trees is not matured enough to produce flowers and fruits. Even though the individual trees flowered massively, the trees did not fruit profusely. This could be due to all opened flowers did not undergo the process of fertilization as the number of existing

pollinators are low. Besides, the opened flowers only last for a few hours and the stigmas were receptive only within a short period.

5.4 Pollen

From the present study, it was found that there are differences in sizes for acetolysed and untreated pollen grains. The acetolysed pollen grains of *V. yeechongii* were bigger than the untreated pollen grains. Nair (1985) reported that acetolysis process can change the original size of the pollen grains.

Erdtman (1952) reported that pollen grains of *Dipterocarpus* and *Monotes* are tricolpate and oblate-subprolate in shape. He stated that the longest axis was 27-65 μm and the exine ornamentation is reticulate. For *V. yeechongii*, the polar-axis for untreated pollens and the acetolysed pollen were ranges from 24.19 μm to 27.76 μm and 28.39 μm to 38.76 μm, respectively. The general pollen morphological characters for *V. yeechongii* were tricolpate and the exine was thick and coarsely reticulate.

Maury and Lugardon (1975) reported the exine structure of nine species from six genera of Dipterocarpaceae, including *Vatica pauciflora* as spherical-subprolate, tricolpate and their surface is smooth. The pollen morphological study conducted by Penny *et al.* (2012) reported that pollen morphology has diagnostic value for distinguishing four dipterocarp species of *Parashorea* and *Shorea* in Sabah.

The pollen grain of *V. yeechongii* is viable almost for two days. From this study, it showed that pollen age 2 hours did not germinate as much as pollen age 4 hours. It might be due to the pollen required a longer period for germination in the media. This reason was supported by results from the viability test for pollen in two different medium which is 15% sucrose + 0.01% boric acid and 10% sucrose + 0.01% boric acid. Pollen aged 4 hours after anthesis showed healthy germination in both media compared to pollen aged 2 hours.

Pollen of *V. yeechongii* produced only one pollen tube in viability test. Pollen grains age 2 hours produced the longest pollen tube in the medium, either 15% sucrose + 0.01% boric acid or 10% sucrose + 0.01% boric acid added. Probably the fresh pollen had more ability to produce longer tube. Media that were used in this viability test also might contributed to the length of tube as the pollen suited well in the certain amount of the sucrose solution.

From the study, the addition of boric acid in distilled water did not show any sign of pollen germination. The pollen grains required sucrose in the media for germination. The addition of 0.01% boric acid in sucrose solution seemed to increase pollen germination and the length of pollen tubes.

As pollen of *V. yeechongii* is viable for almost two days, it can be an advantage to the trees itself as they need pollen from other trees for pollination. Pollen transfer between trees can be done by floral visitor such as *Geniotrigona thoracia, Heterotrigona itama and Tetragonula laeviceps* from the family Apidae as they always visit the newly opened flowers during flowering time. They were able to carry large amount pollen grains at one time and can fly far, thus they may transfered the pollen during visits to other opened flowers of different trees and causing cross pollination between trees. Pollen loads on the body of *H. itama* was the highest (31392 pollen grains) followed by *Lepidotrigona terminata* (23017 pollen grains) and *T. laeviceps* (8015 pollen grains) (Norita *et al.,* 2017).

5.5 Breeding system

It can be seen from this study that *Vatica yeechongii* is an outcrossing species. The pollination experiments showed that cross-pollination between trees resulted in mature fruit set. Based on the index of self-incompatibility (ISI) rating (Zapata & Arroyo, 1978), ISI for *V. yeechongii* is 0. It can be concluded that *V. yeechongii* is completely self-incompatible

and can be classified as outcrossing species. Apomixis did not happen in *V. yeechongii* and this proof that pollination is necessary to produce fruit.

Ghazoul (1997) suggested that *Dipterocarpus obtusifolius* in dry deciduous forests of Thailand are self-incompatible as there is wide spatial separation between the anthers and stigmas in *D. obtusifolius* flowers. Other than that, the ISI rating for *D. otusifolius* is 0.25 and therefore concluded to be self-incompatible. Chan (1977) reported that *Shorea* species within section Muticae (*S. leprosula, S. acuminata, S. lepidota* and *S. hemsleyana*) are highly self-incompatible as there was greater success in fruit set derived from outcrossings than selfings.

Self-pollination experiments showed that *V. yeechongii* was strongly self-incompatible species. It needs pollen from different trees to produced fruit. This result indicated that pollinating agents were also required for successful pollen transfer from the anthers to the active stigma. The overlapping time between anther dehiscence and stigma receptivity may induce self-pollination but the failure of self-pollinated flowers to set fruits indicates chemical and genetic incompatibility.

V. yeechongii flowers have a synchronized maturity and this is necessary for affective cross-pollination. Apart from maximizing opportunities for out-crossing, synchronization in blooming will simultaneously increase the chances for potential gene exchange. Synchronization in flowering and floral maturity is important, especially for outcrossing species with low population densities (Sakai, 2002).

5.6 Pollination

From the observation, it showed that *Vatica yeechongii* is a bee-pollinated plant mainly by insects from family Apidae which is *Geniotrigona thoracia, Heterotrigona itama* and *Tetragonula laeviceps.* These were supported by large amount of pollen grains found on

their bodies such as mouth parts, legs and abdomens as observed through Scanning Electron Microscope (SEM). It is believed that floral visitor from the family Apidae has the highest potential to be a good pollinator and more effective for *Vatica yeechongii* due to their hairy abdomens and legs which were believed to be able to carry more loads of pollens. Bees are known as the most important pollinators for angiosperms as the pollen grains easily stick to the hairs on their thorax and head (Ghazoul, 1997).

The rewards either nectar and/or pollen grains were important characters to attract pollinators. *V. yeechongii* produced large amount of pollen grains per flower. *Apis cerana* and *Trigona laeviceps* collected pollen and nectar for their broods too. Thus, the bees frequently visit the flowers of different or of the same trees during flowering period and thus affecting inter-trees cross pollination (Soepadmo, 1979).

Soepadmo (1979) reported that most Malaysian fruit trees were pollinated by bees species such as *Apis, Trigona* and *Xylocopa*. Pollination in lowland forests is dominated by social bees (mainly *Trigona* and *Apis*), followed by beetles, then other bees and flies (Corlett, 2004). Bees species such as *Trigona* spp. are important pollinators in rain forests and *Apis* sp. are probably more important in cultivated palms (Kiew & Muid, 1989). Appanah (1979) found that *Trigona* bees were important agents in pollination of some tropical forest trees. A social bee (*Trigona*, *Apis* and *Braunsapis*) plays an important role as agent in pollination in the lowland dipterocarp forest compared to the Neotropical forest in Costa Rica dominated by medium to large size of anthophorid bees, where *Apis* is absent (Bawa *et al*., 1985, Kress & Beach, 1994).

Momose *et al.* 1998 found that the flowers of 86 species in 42 families of lowland dipterocarp forest in Sarawak were predominantly visited and pollinated by the genera *Apis* (honey bees), *Trigona* (stingless bees) and *Braunsapis*. *Trigona* species are the major pollinators for *Shorea*

siamensis which one of the four dominant dipterocarp species in the dry deciduous dipterocarp forests of Thailand (Ghazoul & McLeish, 2001).

Beetles are known to visit for rewards such as pollen, nectar or other food; for mating and also for oviposition (Corlett, 2004). Flowers that are primarily pollinated by beetles obviously had a common feature such as a strong odour and variously described as sweet, fruity, musty or foetid in different species (William & Adam, 1994). Dayanandan *et al*. (1990) recorded during a detailed study of *Shorea megistophylla* in Sri Lanka, elaterid beetles consuming pollen, and chrysomelids and scarabeids feeding on the stamen and corolla of the flowers, although large bees appear to be the principals pollinators. In a lowland dipterocarp forests in Sarawak, 20% of the plant species were pollinated by the beetles. Floral tissues, stigmatic secretions and pollen were type of rewards for beetles (Momose *et al.,* 1998). Multiple species of beetles make a visit to feed on petals, pollen and pistils of 20 dipterocarp species from the genera *Hopea*, *Shorea* and *Vatica* that mostly flowered in general flowering periods in the lowland forests of Sarawak (Momose *et al.,* 1998; Nagamitsu *et al*., 1999; Sakai *et al*., 1999). Beetles from the family Chrysomalidae also had the potential to be pollinators for *V. yeechongii*. This is based on the observation on the interactions between beetle bodies and stigma of the flowers. However, the number of beetles observed and frequency of occurrence is low indicating they may not serve as efficient pollinators of *Vatica yeechongii*.

Despite the dominance, the presence of pollen on the body of thrips was very low and this can conclude that thrips were less effective pollinator for *V. yeechongii.* These trees are self-incompatible and had outbreeding system. Besides, the stigma was receptive only for two to three hours, which means that pollinations need to be occur within a short period for successful fruit set. Thrips are minute and energetically limited insects in flights (Appanah & Chan, 1981). It may have a limited chance to conduct pollen transfer during that short

period. Appanah and Chan (1981) reported that floral morphology and flowering behaviour of *Shorea* species (section Muticae) studied in Pasoh are highly adapted for pollination by thrips. It is either to be as the breeding ground and as a source of food. From the observation at a study site in Lambir, Momose *et al*. (1998) reported that thrips were not important pollinators of dipterocarps even they observed the same species that been studied by Appanah and Chan (1981) in Pasoh. This is because the density of thrips per flower was far lower than Pasoh. Instead, Chrysomelids beetles were the major pollinators. Thrips in both populations of *Shorea parvifolia* at Pasoh and Lambir carried less pollen than the beetles and made fewer trips between flowers. In addition, the introduction of thrips to bagged flowers did not increase fruit set while introduction of beetles significantly increase fruit set (Sakai *et al*., 1999).

Flies from the family *Diptera* visited flowers of *V. yeechongii* might be attracted to the sweet scent or fragrance that came from the opened flowers. Although the pollens were found on their abdomen, there is no interaction between the flies and an active stigma. The pollen might be adhered to the abdomen and wings during the flies landed on the petal of the flower. Besides, the number of flies that make a visit are very low, thus it can be concluded the flies were not the pollinators for *V. yeechongii*.

Ants occurred in a great number as they nest on the branch of *V. yeechongii* and they move rapidly on the bracts, inflorescences, and flower. From the observations, the pollens from flowers of *V. yeechongii* were not their main target in search of food as they rarely seen moving inside the open flowers. Besides, the ants carried a little pollen thus makes them less effective for pollen transfer. From the behavioural pattern of ants, it can be concluded that it plays a very minor role in pollen transfer, acting primarily as "nectar thieves" (Rico-Gray, 1980) Altshuler (1999) reported that ants appearance may have indirect impact on pollination through their interactions with other flower visitor, either positive or negative.

5.7 Seed germination

Ng (1991) has reported that *Vatica cinerea and V. lowii* had epigeal germination. Seed of *Vatica yeechongii* favored medium of soil from recreation park mixed with sand for germination. Sand mixed together with the soil helps to improve the soil structure and texture and also improves the porosity of the media. Both combinations which is nutrient from soil and porosity by sand improved seed germination and growth of seedlings. Many seeds did not germinate in soil from recreation park possibly because the structure of the soil is too compact and have more water retention which is not suitable for germination of *V. yeechongii.*

CHAPTER 6: CONCLUSIONS

6.1 General conclusions

From this study, *V. yeechongii* is a dipterocarp species that produced flowers and fruits every year and the trees also had the capability to flower twice a year. Pattern of flowering were about the same for every year where flowering usually starts in February or March. Based on the statistical analysis, the numbers of flowering trees were not significantly correlated with rainfall and mean of the minimum temperature. However, the pattern was predictable as the flowering usually starts in dry months.

The growing process of flower buds from the inflorescence initiation into matured flowers takes about 26 to 32 days. While, the time taken for growing process of young fruits into matured fruits fall takes about 80 to 90 days. Most young fruits abort at early development stages. *V. yeechongii* produced inflorescences in the form of panicles at the axils of leaves and on tree branches.

The flowers of *V. yeechongii* are protandrous and offer pollen as reward. The most common insects that visits these flower were from family Thripidae. However, it is believed that floral visitor from the family Apidae has the highest potential to be a good pollinator and more effective for *Vatica yeechongii* due to their hairy abdomen and legs which enable them to carry more pollens. These *Vatica* is an outcrossing species and completely self-incompatible. Apomixis did not occur in *V. yeechongii* and this proof that pollinating agents is required to produce fruit.

The pollen grains of *Vatica yeechongii* were tricolpate and the exines were thick and coarsely reticulate. Pollen of *V. yeechongii* produced only one pollen tube in the viability test. The pollen grains require sucrose in the media for germination. The addition of 0.01% boric acid in sucrose solution had increased the pollen germination and the lengths of pollen

tubes. The optimum media for pollen germination is 15% sucrose with 0.01% boric acid. The pollen grain of *V. yeechongii* is viable almost for two days.

Seed germination type of *Vatica yeechongii* is epigeal. The fresh seed germinated rapidly and showed high percentage of germination in mixture of soil and sand as media for sowing. The seeds take about two weeks to fully germinate in sand, while seed in other media take about 5 weeks. Seed using soil as media for sowing showed the first appearance of pair leaves in 15 days, 16 days in sand and 18 days in mixture of soil and sand.

6.2 Implications for conservation

From this study, it showed that *Vatica yeechongii* is highly self-incompatible species like other species from genus *Shorea* section Muticae (*S. leprosula, S. acuminata, S. lepidota* and *S. hemleyana*) and has an outbreeding system. Outbreedings were dominance system among fruit tree species in the understorey (Yap, 1976). By reducing size of the population, it will also affect the number of individual trees in that area, which may in time effect and reducing genetic flow among the trees. Thus, *in situ* conservation by giving protection to the forest ecosystem will enable genetic continuously to evolve.

By conducting phenological observations, information regarding the period of time of flowering until mature fruit stage was obtained. Timing for mature fruit to fall can be estimated and conservation measures by *ex situ* can be carried out by collecting the mature fruit and sowing in the nursery. It is to avoid losses in the population as there are a few trees that can produce flower and fruit located on the river bank. This will limit seedling regeneration of that species in that particular area as mature fruit fall into the river.

As the mature fruit germinated well during seed germination experiment, it can be used for reforestation by planting the seedling at suitable locality that suits well with characters of this species. However, to collect bulk of mature fruits rely upon the general

flowering periods, which is difficult to predict. Thus, long term phenological observations need to be done in order to understand more and to detect physiological causes of general flowerings.

From the estimation of number of fruit, it shows that the number of mature fruit is far lower from the number of flowers produced. Cross pollination experiment carried out during compability study resulted in fruits. The pollination technique employed in this study could be further developed and apply onto flowering trees as it will help to increase the number of fruit set.

V. yeechongii will face the risk of extinction if conservation measures at the Sungai Tekala recreational forest park are not implemented properly. Population of *V. yeechongii* is found on gentle earth banks beside stream near the public campsite. As this area is designated for recreational purposes, clearing of the undergrowth and cleaning are done daily to maintain cleanliness. Even though the population lies in a protected area, recreation activities and regular cleaning will negatively impact the regeneration and survival of the population. The population at Setul FR is located in a very small isolated fragment forest margin, thus edge effects are likely to severely impact the population in the long term. Data and information on the flowering pattern, floral biology, pollination and breeding systems are now ready to be used to develop a conservation action plan for *V. yeechongii*. Studies at the molecular level are recommended for future work in order to understand the population genetics of *Vatica yeechongii*.

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APPENDICES

Appendix A

Preparation of specimens for scanning electron microcopy (SEM) :

Critical Point Drying (CPD) Procedures

1. Specimens were soaked in glutaraldehyde + Sorensen's phosphate buffer (SP) (ratio 1:1) for one hour
2. The solution was then poured away and changed to SP + distilled water (1:1)
3. The next change was to osmium + water (1:3) for 14 hours in a fume hood
4. The specimens were soaked for 15 minutes in each solution listed below in the fume hood :
    a. Water, 10%, 20%, 30%, 40%, 50%, 60% and 70% ethanol
5. The specimens were taken out of the fume hood and soaked for 15 minutes in 80%, 90% and 100% solutions each.
6. Then the specimens were soaked in 100% ethanol + 100% acetone solutions for 20 minutes, each at the ratio of 3:1, 1:1 and 1:3.
7. The specimens were soaked again in 100% acetone for 20 minutes, in four exchanges of fresh solutions.
8. Finally, the specimens were gold coated using sputter coater.

Appendix B

Pollen grains acetolysis (Erdtman, 1969)

1. Fresh pollen was placed in glass centrifuge tubes
2. Five millimeters of acetolysing mixtures of 9:1 of pore acetic anhydride and sulphuric acid was added to the tubes. The mixtures was stirred with a glass rod and kept in a 70 ºC water-bath until boiling point
3. When the mixtures turned dark brown, the tubes were removed from the water-bath and left to cool. The mixtures was filtered through conical sieves into new plastic centrifuge tubes.
4. 100% glacial acetic acid was added to balance the tubes on a balance. The pollen was then centrifuged at 700 rotation per minute (r.p.m.) for 15 minutes.
5. The glacial acetic acid was decanted off slowly and the sediment washed in a mixture of 3:1 distilled water and 95% alcohol respectively to remove the acid completely before recentrifuging. This process was repeated three times.
6. After washing, the solution was decanted off and the residue air-dried in an oven at 40 ºC for a few days.
7. The dried acetolysed pollen was then mounted in glycerine jelly stained with Safranin and sealed with wax.

Appendix C

Sitting drop culture for *in vitro* germination of the pollen (Shivanna and Rangaswamy, 1992).

1. Two drops of 5% + 0.01% boric acid (b.a.) solution were placed on a concave slide. Then, a small amount of the pollen was dispersed homogeneously in the drop of solutions with a needle
2. The concave slide was kept in a humidity chamber moistened with distilled water, and raised on two bars of staples at room temperature (27-32 ºC) for three hours.
3. Steps 1 and 2 were repeated with distilled water, 10% and 15% sucrose, each with 0.01% boric acid added, and 5%, 10% and 15% sucrose solutions without boric acid (b.a).
4. A drop of formalin-acetic acid-alcohol (FAA) was added to every culture at the end of the incubation.
5. The culture was scored for pollen germination under a microscope. A pollen grain was considered germinated when the length of its tube was at least twice the diameter of the grain.

$$\text{Pollen germination} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Appendix 1

A. Spearman's Rho Correlation (two-tailed) (using SPSS Ver. 23) analysis of number tree flowering mean of minimum temperature and rainfall in Sungai Lalang FR.

Correlations

			Rainfall	Mean_min_ temperature	No_tree_flowering_ sglalang
Spearman's rho	Rainfall	Correlation Coefficient	1.000	.131	-.136
		Sig. (2-tailed)	.	.446	.430
		N	36	36	36
	Mean_min_temperature	Correlation Coefficient	.131	1.000	.011
		Sig. (2-tailed)	.446	.	.949
		N	36	36	36
	No_tree_flowering_sgtekala	Correlation Coefficient	-.136	.011	1.000
		Sig. (2-tailed)	.430	.949	.
		N	36	36	36

B. Spearman's Rho Correlation (two-tailed) (using SPSS Ver. 23) analysis of number tree flowering mean of minimum temperature and rainfall in Setul FR.

Correlations

			No_tree_flowering_setul	Mean_min_temperature	Rainfall
Spearman's rho	No_tree_flowering_setul	Correlation Coefficient	1.000	-.019	-.113
		Sig. (2-tailed)	.	.910	.513
		N	36	36	36
	Mean_min_temperature	Correlation Coefficient	-.019	1.000	-.319
		Sig. (2-tailed)	.910	.	.058
		N	36	36	36
	Rainfall	Correlation Coefficient	-.113	-.319	1.000
		Sig. (2-tailed)	.513	.058	.
		N	36	36	36