UNIVERSITI PUTRA MALAYSIA

SEED MATURITY, GERMINATION, DORMANCY AND STORAGE OF *Melicope lunu-ankenda* (Gaertn.) Hartley HARVESTED AT DIFFERENT MATURITY STAGES

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstracts of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

SEED MATURITY, GERMINATION, DORMANCY AND STORAGE OF *Melicope lunu-ankenda* (Gaertn.) Hartley HARVESTED AT DIFFERENT MATURITY STAGES

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August 2018

Chairman : Professor Uma Rani Sinniah, PhD
Faculty : Agriculture

Melicope lunu-ankenda, locally known as "Tenggek Burung" or "Cabang Tiga" is a tree species belonging to the Rutaceae family. *M. lunu-ankenda* is traditionally used to relieve symptoms of fever, revitalize the body and reduce high blood pressure. Studies have shown that the extract of *M. lunu-ankenda* contains antioxidants and anti-inflammatory properties. The fresh seeds have difficulty in germinating and takes more than 7 months to germinate. Studies need to be carried out to determine the cause of this delay in germination of *M. lunu-ankenda* seeds.

For the first part, flower tagging was carried out in the first week of June 2014. Fruits were collected at random from the labeled panicles during each harvest (42, 56, 63, 70 and 77 days after tagging). Shape, size, colour and characteristics of the seed coat was documented. Seeds were tested for fresh weight, dry weight and moisture content (%). 50 seeds from each stage were germinated on 5 different types of media (Sand, Soil Mixture, Peat Soil, Peat Moss and Filter Paper). For the second part of the study, mature seeds harvested at day 77 after flowering were subjected to physical and chemical treatments as follows: (1) Soaked in distilled water for 12, 24 and 36 hours. (2) Scarified using sand paper for 1, 2 and 3 minutes. (3) Soaked in 98% Sulfuric acid for 10, 15 and 30 seconds. (4) Soaked in 200 ppm, 400 ppm and 600 ppm concentration of GA_3 for 10 minutes. (5) Soaked in 0.2%, 0.4% and 0.6% concentration of KNO_3 for 10 minutes. Imbibition rate for scarified seeds were tested in comparison to fresh seeds. Seeds were then stored in different temperatures (29 ºC, 15 ºC and 10 ºC) for six months. Germination percentage were recorded every two months after storage.

Results for the first part showed that the the maximum dry weight per 100 seed (1.523 g) was recorded at 70 days after anthesis where fruit pods were dark green in colour while the seeds were black and fully developed. Moisture content percentage of seeds during dehiscence was recorded at 8.6%. Seeds obtained on day 70 and 77 showed the ability to germinate after 90 days with a percentage of 28.5% and 61.5%, respectively on peat soil. For the second part of the experiment, seeds scarified using sand paper for two minutes showed the highest germination percentage with 83%. Germination starting time was also reduced from 90 days to 20 days after sowing. Scarified seeds had a significantly higher imbibition rate in the first 12 hour compared to normal seeds. When the imbibition period reached 12 hours, scarified seeds achieved maximum water imbibition (0.381 g) compared to normal seeds which only imbibed 0.035 grams of water. After 6 months of storage, highest germination percentage and germination index was recorded when seeds were stored at 29°C, 79.5 % and 1.374, respectively.

In conclusion, *Melicope lunu-ankenda* seeds exhibit physical seed dormancy that can be overcome by mechanical scarification of the seed coat. Of all the dormancy breaking techniques tested, it is recommended that the seeds are scarified using sand paper for two minutes. The most suitable media to germinate the seeds is peat soil. Seeds must be harvested at 77 days after flowering in order to obtain the highest germination percentage. Since mature seed naturally has a moisture content of 8.6% and can be stored until 6 months, seeds storage behaviour can be catagorised as orthodox.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KEMATANGAN, PERCAMBAHAN, KEDORMANAN DAN PENYIMPANAN BIJI BENIH *Melicope lunu-ankenda* (Gaertn.) Hartley DITUAI PADA TEMPOH KEMATANGAN YANG BERBEZA

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Melicope lunu-ankenda atau lebih dikenali sebagai Tenggek Burung atau Cabang Tiga merupakan pokok daripada keluarga Rutaceae yang digunakan secara tradisional untuk meredakan deman, menyegarkan tubuh dan menurunkan tekanan darah tinggi. Penyelidikan yang dijalankan telah membuktikan bahawa ekstrak *M. lunu-ankenda* mengandungi antioksida dan anti-radang. Benih segar mempunyai masalah untuk bercambah dan memerlukan lebih dari 7 bulan untuk bercambah. Kajian perlu dijalankan bagi mengenal pasti punca percambahan yang lewat dalam biji benih *M. lunu-ankenda.*

Bagi bahagian pertama, penandaan bunga dijalankan terhadap 20 panikel dari 4 pokok dan dilabel pada hari pembungaan pada minggu pertama Jun 2014. Buah dikutip secara rawak daripada panikel yang ditanda (42, 56, 63, 70 dan 77 selepas ditanda). Bentuk, saiz, warna dan sifat kulit biji benih dicatatkan. Biji benih ditimbang bagi mencatatkan berat basah, berat kering dan peratus kelembapan. Biji benih daripada peringkat berbeza tersebut kemudiannya dicambah di atas 5 media yang berbeza (Pasir, Campuran Tanah, Tanah Gambut, Peat Moss dan Kertas Turas). Bagi bahagian kedua, biji benih dikutip pada hari 77 selepas pembungaan (peringkat matang) dan telah diuji dengan rawatan fizikal dan kimia seperti berikut: (1) Rendaman dalam air suling selama 12, 24 dan 36 jam. (2) Dikikis menggunakan kertas pasir dengan masa 1, 2 and 3 minit. (3) Direndam di dalam 98% Asid Sulfurik dengan masa for 10, 15 and 30 saat. (4) Direndam di dalam 200 ppm, 400 ppm dan 600 ppm kepekatan GA3 selama 10 minit. (5) Direndam di dalam KNO_3 dengan kepekatan 0.2%, 0.4% dan 0.6% selama 10 minit. Kadar serapan air oleh benih yang dikikis diuji berbanding benih segar. Benih matang kemudiannya disimpan di dalam suhu yang berbeza (29 ºC, 15 ºC dan 10 ºC) untuk 6 bulan. Peratus percambahan

dicatatkan setiap 2 bulan.

Keputusan bahagian pertaman menunjukkan berat kering tertinggi (1.523 g) dicatatkan pada hari ke-70 selepas pendebungaan di mana pod buah berwarna hijau gelap dan warna biji benih berwarna hitam dan telah masak. Peratus kelembapan semasa benih masak adalah 8.6%. Biji benih yang dikutip pada hari ke-70 dan 77 mempunyai kebolehan untuk bercambah selepas 90 hari dengan 28.5% dan 61.5% masing-masing di atas tanah gambut. Bahagian kedua menunjukkan biji benih yang dikikis menggunakan kertas pasir selama 2 minit menunjukkan percambahan yang paling tinggi dengan peratus percambahan 83%. Masa permulaan percambahan juga telah dikurangkan daripada 90 hari ke 20 hari berbanding benih segar. Merendam benih di dalam air suing selama 36 jam juga memberikan peratus bercambahan sebanyak 23%. Biji benih yang dikikis didapati mempunyai kadar serapan air yang lebih tinggi dalam tempoh 12 jam pertama. Biji benih yang dikikis menyerap air secara maksimum pada tempoh 12 jam (0.381 g) berbanding benih segar (0.035 g). Setelah disimpan selama 6 bulan, peratus percambahan dan indeks percambahan tertinggi dicatatkan oleh biji benih yang disimpan pada suhu 29°C, 79.5 % dan 1.374, masing-masing.

Secara kesimpulan, biji benih segar *M. lunu-ankenda* mempunyai kebernasan benih yang bercampur dan perlu dipisahkan dengan menggunakan kaedah rendaman air. Biji benih *M. lunu-ankenda* menunjukkan kedormanan fizikal yang boleh diatasi dengan melukakan kulit biji benih dengan kaedah mekanikal. Kikis kulit biji dengan kertas pasir selama 2 minit merupakan kaedah terbaik untuk memecahkan kedormanan benih. Media percambahan yang paling sesuai ialah tanah gambut. Biji benih perlu dikutip pada peringkat matang (77 hari selepas pembungaan) bagi mendapatkan peratus percambahan yang tinggi. Biji benih matang mempunyai peratus kelembapan 8.6% secara semulajadi serta boleh disimpan sehingga 6 bulan, sifat simpanan benih boleh dikategorikan sebagai ortodoks.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

MARDI	Malaysian Agricultural Research and Development Institute
g	gram
MGT	Mean Germination Time
mm	millimeter
cm	Centimeter
GI	Germination Index
DAA	Day After Anthesis
DAT	Day After Tagging
PM	Physiological Maturity
GA3	Gibberellic Acid
KNO3	Nitric acid
ppm	Parts per million

CHAPTER 1

INTRODUCTION

SThe herbal industry is growing at a very fast pace globally. Projected to reach a market worth USD 250 billion by 2020 (Global Industry Analysts, 2012), this industry is driven by the growing adoption of healthy living and preventive care as a safe and effective strategy to improve health and well-being. The United States of America and China are the biggest players in the herbal industry. According to the Council for Responsible Nutrition in 2012, 23% of an approximate population of 314 million use herbs and botanicals to prevent or cure various ailments in the United States. In China, over 1.3 billion people depend on herbs as a primary healthcare needs which has led to an estimated $11 billion dietary supplement market. Experts predict that China will become the largest global producer and consumer by 2020 (World Nutraceutical Ingredients: Industry Study, 2012). Malaysian herbal industry is expected to expand from RM 7 billion in 2010 to an estimated RM 29 billion by 2020, a total of 15% increment per year. The Malaysian household consume herbal products at 73%, 7% short of the estimation done by the World Health Organisation (WHO) in 2012 for developing countries (Farizah *et al.*, 2015).

Malaysia in known as one of the 17 megadiversity countries in the world. It is estimated that Malaysia has 15,000 known plant species, with 3,700 species known to be useful and 2,000 species are known to have medicinal value. However, only around 50 plant species are commonly used in traditional medicine and mostly are consumed as herbal drinks and salads. Agriculture, being one of the National Key Economic Areas (NKEA) of Malaysia, emphasizes on herbal products. Entry Point Project No.1 under Agriculture NKEA is producing high value herbal products. One of the herbal products that is getting attention is *Melicope lunu-ankenda*, which has various medicinal value including anti-diabetic and antibiotic properties.

M. lunu-ankenda, locally known as "Tenggek Burung" or "Cabang Tiga" is a tree species belonging to the Rutaceae family. Consumed fresh as salads, Tenggek Burung is traditionally used to relieve symptoms of fever, revitalize the body and reduce high blood pressure. Tenggek Burung has very high medicinal value. Leaves of Tenggek Burung were recently reported to contain *O*-prenylated flavonoid (OPF) which is a compound that has anti-diabetes activity against type-2 diabetes mellitus (George *et al.*, 2015). Studies have also shown that Tenggek Burung leaves have anti-quorom sensing properties which can help in curing patients infected by antibiotic-resistant bacteria (Tan *et al.*, 2012). Tenggek Burung leaves contains evodione and leptonol which helps reduce fever and inflammation (Johnson *et al.*, 2010). The leaves also have bacteriostatic, fungicidal activity and acts as a natural anti-oxidant (Johnson *et al.*, 2010). Due to the increased potential of this species, the production of planting materials of an excellent quality is necessary.

The Malaysian Agricultural Research and Development Institute (MARDI) has been producing seedlings of *M. lunu-ankenda* since the year 2012. Starting with 200 seedlings in 2012, the production was increased to 1,200 by the year 2016 due to its popular demand (MARDI, 2017). Production was done by collecting seedlings which grow near mother plants and transplanting them into polybags. The conventional method of propagating *M. lunu-ankenda* is through seeds. However, the fresh seeds have difficulty in germinating and takes more than 6 months to germinate in favourable conditions. Most seeds from *Melicope* sp. will take up to 7 months to germinate in cleaned, kept moist and well-lit conditions (Burrows, 1996). When fresh seeds of *Melicope* sp. was germinated, only 17% we able to germinate (Martyn *et al.*, 2009). Generally, seeds from Rutaceae family are known to have germination difficulties (Auld, 2001). The seed coat of plants from Rutaceae family which sometimes are thick and has a waxy coating which may act as a barrier to the imbibition process (Auld, 2001). *Melicope* seeds often have thick coats which may be involved with the germination delay and hence, a test on the effects of nicking the coats is required for this species (Burrows, 1996).

Understanding germination pattern and behavior of seeds is important as seeds directly linked to plant generation. Seeds are very critical for plants to retain their existence until they encounter a favourable environmental conditions to complete their life cycle. The germination timing is another important aspect for the plant survival. Because, seeds germinated during drought or unfavorable conditions may not survive or their generation rate will be diminished. The seed dormancy is a type of control methods for seeds to withstand such harsh environmental conditions.

Seed dormancy is a barrier for planters to mass propagate plant species. A study on the seed germination behavior and seed dormancy of *Melicope lunu-ankenda* is important and beneficial for mass propagation and to adopt good agronomic practices. Very limited data is available on the germination requirements and dormancy breaking of *M. lunu-ankneda*.

Land scarcity is lately becoming a serious issue. Land usage for agriculture (including oil palm and rubber) in Malaysia is currently at 36.8% (Statistics Malaysia, 2016) and is predicted to shrink due to development of housing estates and industrial use. Conserving plants has become a priority to protect useful plant species. Conserving plants can be divided into two categories, in-situ and ex-situ. In in-situ conservation, plants are conserved in their original habitat, while ex-situ method involves the preservation of components of biological diversity outside their natural habitats. According to Food and Agriculture Organization of the United Nations (FAO) in 1993, ex-situ conservation is most widely preferred practice due to the alarming rate of deforestation. Since Tenggek Burung produces numerous seeds, using seed bank as a method to store and protect this species is the most suitable way. However, to store the seeds, germinability of the seeds must first be established.

The study was conducted to determine the type of dormancy that is affecting *M. lunu-ankenda* seeds and discover suitable methods of breaking dormancy of the seeds. This study also aims to determine the type of seed storage behavior and henceforth, establish a method to store the seeds. Considering the above facts, the following studies were carried out to establish a suitable technique to mass propagate *M. lunu-ankenda*.

The objectives of this study include:

1. To study the germination pattern of *M. lunu-ankenda* seeds harvested at different maturity stages and using different media.
2. To determine the class of dormancy affecting and establish a suitable dormancy breaking method *M. lunu-ankenda* seeds.
3. To determine the type of seed storage behaviour and establish a method to store the seeds.

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