UNIVERSITI PUTRA MALAYSIA

FRUIT MATURITY, SEED TREATMENT AND STORAGE OF COFFEA LIBERICA HIERN

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FP 2018 103

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements of the Degree of Master of Science

August 2018

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

FRUIT MATURITY, SEED TREATMENT AND STORAGE OF *Coffea liberica* HIERN

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August 2018

Chairman : Professor Uma Rani Sinniah, PhD
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Coffee seeds obtained through conventional methods performed poorly in the field with very slow and asynchronous germination. Harvesting at the right stage of maturity may result to improve the performance of seeds during the rootstock development. This study was carried out to determine the suitable fruit maturity stages for seeds to be used in seedling establishment, to determine the effect of various seed pretreatments to obtain a rapid germination rate and to establish an appropriate drying and storage method for *Coffea liberica*. The first part of study was conducted to assess the germination ability of seven different *C. liberica* clone seeds namely MKL 1, MKL 2, MKL 3, MKL 4, MKL 5, MKL 6 and MKL 7. The results revealed that seeds from MKL 5 had the highest germination percentage (GP) of 93%, germination index (GI) of 0.97 and mean germination time of 29 days, while seeds from MKL 3 had the lowest with 70% germination percentage (GP), 0.62 germination index (GI) and mean germination time (MGT) 35 days.

The second part of the study determines the suitable fruit maturity stage for seedling establishment. From this part, the effect of maturity stages on germination and seedling establishment for *C. liberica* was studied using seeds from four stages of maturity. Seeds were extracted from fruits harvested at immature unripe [210 days after anthesis (DAA)], mature unripe (260 DAA), mature ripe (310 DAA), and overripe (360 DAA) stages. It was observed that the germination percentages between all the maturity stages were significantly different. Seeds of MKL 5, MKL 6 and MKL 7 were not able to germinate when harvested at immature unripe stage at stage 3. The highest germination percentage and germination index among all the three clones was obtained when seeds were collected at stage 3 (mature ripe). MKL 5 had the highest germination percentage with an average of 95%, MKL 6 gave 78% and MKL 7 was 84%. Fruits harvested at mature ripe stage showed better

performance in all parameters evaluated. Therefore, harvesting of fruits at mature ripe stage is suggested to obtain faster and uniform growth of coffee seedlings.

For the third part, the effect of seed treatment on the germination of *C. liberica* was studied. Seeds were grouped and treated with $KN0_3$ and GA_3. The treatments included untreated seeds (control), seeds soaked with $KN0_3$ (0.2%, 0.4% and 0.6% for 12 and 24 hours) and GA_3 (200 ppm, 400 ppm and 600 ppm for 12 and 24 hours). The effect of different growth condition (continuous light, continuous darkness and with 12h:12h light:dark) was also evaluated. The effect of growth condition tested as the third part of the study showed that *C. liberica* seeds germinated slowly when sown with continuous light (24 hrs light). It showed only 47.5% germination percentage, 0.25 germination index and required a mean germination time of 55.2 day. A significantly higher germination percentage was achieved at 25°C with 12-hour light and 12 hourr dark and 75% humidity. Seeds soaked in GA_3 200 ppm for 12 hours and KNO_3 0.4% for 24 hours did not significantly affected the germination percentage as compared to controls. However, they had significantly a higher germination index in comparison to control treatments.

For the last part of this study, the seeds were then desiccated to determine the effect of seed moisture content on germinability of *C. liberica*. Germination percentage was reduced when the seeds were dried to the lowest moisture contents. Fruits from clone MKL 5 were dried out under the sunlight for three weeks until the moisture content reached 10%. The bag was sealed tightly to prevent moisture penetration, labelled and stored at 10°C, 15°C, 25°C and 29°C. Polyethylene bags stored at different temperatures were retrieved every month to test the germination rate. Germination percentage of seeds with 20% moisture content were higher with an average of 79%, 73% stored at 15°C in the second and fourth month. Seeds deterioration rate was higher at 29°C. The deterioration may be caused by the growth of fungi which is known to affect the seeds. Seeds containing 10% moisture content failed to germinate starting from the sixth month of storage. The germination percentage for this *C. liberica* seeds were higher as seed were dried to the lower moisture content irrespective of the temperature regimes.

In conclusion, the most suitable seeds to be used for coffee seedling production are from the clone MKL 5 which were harvested at stage 3 (mature ripe) which has resulted with 93% germination rate. Chemical treatments did not increase germination percentages, but condition under 12-hour light and 12-hour dark played an important role of *C. liberica* growth by reducing mean germination time to 32.15 days compared to 55.22 days. For storage, seeds should be dried to 20% moisture content and stored at 15°C up to six months.

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

KEMATANGAN BUAH-BUAHAN, RAWATAN BIJI BENIH DAN PENYIMPANAN *C. liberica* HIERN

Oleh

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Biji kopi yang diperolehi melalui kaedah biasa didapati mempunyai prestasi pertumbuhan yang lemah di lapangan dengan percambahan yang perlahan dan tidak seragam. Penuaian pada peringkat kematangan yang betul boleh meningkatkan kualiti dan prestasi biji benih bagi penyediaan anak pokok. Kajian ini dijalankan untuk menentukan peringkat kematangan buah yang sesuai untuk biji benih digunakan dalam pengeluaran anak pokok, untuk mengenalpasti kesan pelbagai rawatan awal benih bagi mendapatkan percambahan cepat dan untuk menetapkan kaedah pengeringan dan penyimpanan *Coffea liberica* yang sesuai. Bahagian pertama kajian telah dijalankan untuk menilai keupayaan percambahan tujuh klon klon Liberica yang berbeza iaitu MKL 1, MKL 2, MKL 3, MKL 4, MKL 5, MKL 6 dan MKL 7. Benih dari MKL 5 mempunyai peratusan tertinggi percambahan (GP) sebanyak 93%, indeks percambahan (GI) sebanyak 0.97dan mencapai min masa percambahan 29 hari manakala benih dari MKL 3 mempunyai yang terendah sebanyak 70% peratusan percambahan (GP), 0.62 indeks percambahan (GI) dan min masa percambahan (MGT) 35 hari.

Bahagian kedua kajian menentukan peringkat kematangan buah yang sesuai untuk penghasilan anak benih. Dari bahagian ini, kesan tahap kematangan ke atas pembiakan dan pembentukan anak benih untuk *C. liberica* diteliti di mana benih dari buah yang mewakili empat peringkat kematangan. Benih telah diekstrak daripada buah-buahan yang dituai pada peringkat tidak matang [210 hari selepas anthesis (DAA)], matang tidak masak (260 DAA), masak matang (310 DAA), dan tahap tua/terlebih masak (360 DAA). Peratusan percambahan di antara semua peringkat kematangan juga jauh berbeza. Benih MKL 5, MKL 6 dan MKL 7 tidak dapat bercambah apabila dituai pada peringkat tidak matang. Peratusan percambahan tertinggi dan indeks percambahan di antara ketiga-tiga klon diperoleh apabila benih

dikumpulkan pada peringkat 3 (masak matang). MKL 5 mempunyai peratusan percambahan tertinggi dengan purata 95%, MKL 6 memberikan 78% dan MKL 7 adalah 84%. Buah-buahan yang dituai pada tahap masak matang menunjukkan prestasi yang lebih baik dalam semua parameter yang dinilai. Oleh itu, penuaian buah-buahan pada peringkat masak matang adalah dicadangkan untuk mendapatkan pertumbuhan anak benih kopi yang lebih cepat dan seragam.

Untuk bahagian ketiga, kesan rawatan benih pada percambahan *C. liberica* telah dikaji. Benih dikumpulkan dan dirawat dengan $KN0_3$ dan GA_3. Rawatan adalah benih yang tidak dirawat (kawalan), benih direndam dengan $KN0_3$ (0.2%, 0.4% dan 0.6% untuk 12 dan 24 jam) dan GA_3 (200 ppm, 400 ppm dan 600 ppm selama 12 dan 24 jam). Kesan keadaan pertumbuhan yang berbeza (25°C dengan cahaya berterusan, 25°C dengan kegelapan berterusan dan 25°C dengan 12h: 12h cahaya: gelap) juga dinilai. Kesan keadaan pertumbuhan yang diuji sebagai bahagian ketiga kajian menunjukkan bahawa biji *C. liberica* lambat untuk bercambah apabila disemai pada suhu 25°C dengan cahaya berterusan (24 jam cahaya) dan ia menunjukkan daripada peratusan percambahan 47.5%, indeks percambahan memberikan 0.25 dan masa percambahan bermakna adalah 55.2. Peratusan percambahan yang ketara dicapai pada 25°C dengan cahaya 12 jam dan 12 jam gelap dan kelembapan 75%. Benih yang direndam dalam GA_3 200 ppm selama 12 jam dan KNO_3 0.4% selama 24 jam tidak menjejaskan peratusan percambahan (GP) berbanding kawalan. Walau bagaimanapun, mereka mempunyai indeks percambahan yang lebih tinggi (GI) daripada kawalan.

Untuk bahagian akhir kajian ini, biji benih kemudian dikeringkan untuk menentukan kesan pertumbuhan ke atas *C. liberica*. Peratusan percambahan mengalami pengurangan apabila benih dikeringkan ke kandungan kelembapan yang paling rendah. Buah-buahan dari klon MKL 5 telah dikeringkan di bawah cahaya matahari selama tiga minggu sehingga kandungan lembapan mencapai 10%. Beg tersebut diikat dengan ketat untuk mencegah kelembapan, dilabel dan disimpan pada suhu 10°C, 15°C, 25°C dan 29°C. Beg polietilena yang disimpan pada suhu yang berbeza telah diambil setiap bulan untuk menguji kadar percambahan. Perubahan dalam peratusan percambahan benih dengan kandungan lembapan sebanyak 30%, 20% dan 10% yang disimpan pada 10°C, 15°C, 25 °C dan 29°C. Peratusan percambahan dengan kandungan kelembapan 20% lebih tinggi dengan purata 79%, 73% disimpan pada suhu 15°C dalam simpanan kedua dan keempat. Penyelidikan lain juga menunjukkan bahawa penyimpanan benih optimum untuk kopi adalah 15°C (Ellis *et al.*, 1990, 1991). Kadar kemerosotan benih adalah lebih tinggi pada 29°C. Kemerosotan mungkin disebabkan oleh kulat yang diperhatikan wujud dalam beg polietilena. Benih yang mengandungi kandungan kelembapan 10% gagal berkecambah bermula dari bulan keenam penyimpanan. Peratusan percambahan untuk biji *C. liberica* ini lebih tinggi kerana benih dikeringkan ke kandungan lembapan yang lebih rendah tanpa mengira rejim suhu.

Sebagai kesimpulan, biji kopi yang paling sesuai untuk digunakan untuk pengeluaran benih kopi adalah dari klon MKL 5 yang dituai pada peringkat 3 (masak matang) yang menghasilkan 93% percambahan. Rawatan kimia tidak meningkatkan peratus percambahan, tetapi fotoperiod 12 jam cahaya dan 12 jam gelap memainkan peranan penting pertumbuhan *C. liberica* dengan mengurangkan masa percambahan min kepada 32.15 berbanding 55.22. Untuk tujuan penyimpanan, benih perlu dikeringkan hingga 20% kandungan lembapan dan disimpan pada suhu 15°C sehingga enam bulan penyimpanan.

ACKNOWLEDGEMENTS

"In the name of Allah S.W.T. for His most gracefulness and kindness,"

First and foremost, I would like to express my utmost appreciation to the beloved chairperson of my supervisory committee, Professor Dr. Uma Rani Sinniah for her help, advice, guidance and support during the course of the study. Her patience, friendly and kindness encouragement gave me the confidence to overcome all the problems in this research. My sincere thanks also go to the members of my supervisory committee, Professor Dr. Adam Puteh for the idea and support to the completion of this research.

A special thanks to my institute, Malaysian Agriculture and Development Institute (MARDI) for giving me the opportunity to further my study and financing it with the scholarship. This opportunity gave me more knowledge and spirit to work harder for the institute and continue to give services to government and citizen of Malaysia. I am also very grateful to Universiti Putra Malaysia for accepting me as the post-graduate student and providing all the facilities for this study. I would like to thank all the staff especially from Crop Science Department, Faculty of Agriculture for their help and contributions.

Special thanks to my husband Ariff Merican Din Merican, my beloved parents Abd Rahman Abd Hamid and Salmah Che Doi, my siblings Mohd Faridzul, Faezah Salfarina and Faezah Salfazira for being very supportive, understanding and encouraging throughout my study and not forgetting all of my lovely friends especially, Muhammad Najib, Anis, Khairul Anuar, Zaitialia Mohlisun, Amyita Witty Ugap and Dr Juju Nakasha.

My gratitude also goes to the staff and workers at Pusat Bank Gen dan Biji Benih MARDI, Serdang for their assistance in the field and in the laboratory. I would also like to express my appreciation to Kak Anis for their help and kind support. Thank you to my mentors, Dr. Mohammed Selamat Madom and Hj. Mohd Shaib Jaafar for encouragement and knowledge. Thank you also to Datuk Dr. Mohamad Roff bin Mohd Noor for your concern, help and support.

Appreciation also goes to all of my lab mates from Cryopreservation and Seed

Technology Laboratory especially to Greetha, Dr Juju, Ezzah and Dr Kumara for sharing their knowledge and opinion throughout my research.

Last but not least, I would like to express my deepest appreciation to my parents and other family members for their love, support, and encouragement. I would like to express my deepest thanks to my husband spirit, support and prayers, without which the study would not have been successfully completed.

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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MARDI	Malaysian Agricultural Research and Development Institute
MKL	MARDI Klon Liberica
g	gram
mm	millimeter
cm	Centimeter
m	Meter
GI	Germination Index
DAA	Day After Anthesis
PM	Physiological Maturity
GA_3	Gibberellic Acid
KNO_3	Nitric acid
ppm	Parts per million
RH	Relative Humidity
Ha	hectare
%	Percentage
°C	Degree Celcius
GP	Germination Percentage
GI	Germination Index
MGT	Mean Germination Time
DW	Dry weight
FW	Fresh weight

CHAPTER 1

INTRODUCTION

Nowdays, coffee is the most sought after commodity in the developed world. Coffee is very important in the global economy because, it is one of the main valuable products in the global market. Coffee is placed in the family, Rubiaceae and the genus, *Coffea*. Coffee can be catagorised as a shrub native to the tropical regions of Asia and the southern parts of Africa. *C. liberica* plant is native west tropical parts of Africa (Gomez *et al*., 2009). In South East Asia countries, *C. liberica* is planted in Malaysia, Indonesia and The Philippines, some parts in Surinam and also in Guyana regions (Lim, 2014). According to Lim (2014), *C. liberica* is a species that favours warm tropical climate growing in low land montane rain-forests and is commonly grown in altitudes up to 1,200 m. The major species of coffee planted in Malaysia include Liberica with 73% areas planted and Robusta at 27%, while the highland areas are planted with Arabica in a negligible amount (MARDI, 1984).

Liberica type coffee (*C. liberica* Bull ex Heirn.) and Robusta type coffee (*Coffea canephora* Pierre ex Froehner.) both indigenous to the African continent were introduced to the peninsular Malaysia in the 19th century (Davis *et al*., 2006). Both species are suitable to be cultivated in the lowland areas of the warm tropical climate of this country. The request for better planting materials by the Ministry of Agriculture (Anon. 1985) has been partially fulfilled by MARDI through the release of polyhybrid planting materials (MKL 1) in 1992 (Razak Rashid 1992; Zulkifli 1992) and three clones (MKL 2, MKL 3 and MKL 4) in 1995 (Anon. 1995a, b). The acceptance of these recommended planting materials is encouraging. Until 2004, about 1.7 tonnes of seeds and 75,000 seedlings of polyhybrids (MKL 1) and 36,000 clonal seedlings and 19,000 scions of clonal materials have been sold by MARDI. In a working paper on action plans for food production in the 8th and 9th Malaysian National Plans (Anon. 2000), it was stated that the government should continue to give support to ensure that the local coffee industry in the country is sustainable.

Presently, germinated seed is the key component of planting material for cultivation of coffee. The germination pattern of ripe coffee seeds (dried) is very slow and erratic. This causes difficulty in obtaining seedlings that are required for coffee crop establishment for coffee production. For commercial planting, grafted seedlings are used which requires the production of rootstock. Finding coffee rootstocks for commercial production is troubled by slow germination. The capability to germinate for coffee seeds can differ a lot and largely dependent on the maturity stage at harvest. Even though some have the capability to germinate before reaching physiological maturity, the potential of seeds to obtain maximum germination usually is achieved at physiological maturity.

In order to cultivate the plants in a large scale, high quality planting material is needed. A study on seed maturity stage needs to be done to indicate the best harvesting time with good quality seeds. According to Olasoji (2012), studies on seed development and physiological maturity is vital because seeds should be collected at a proper period in order to guarantee seed quality especially their viability and vigor. Seed quality must be at high level during its physiological maturity (Baskin, 1995). Physiological maturity of seeds is achieved when it attains its dry weight maximum at seed development stage (Harrington, 1972). The physiological maturity occurs before harvest maturity or before the seed desiccates on the mother plant. Different plants species have different seed moisture content and seed development which cause the seeds to reach physiological maturity at different time. Some seeds have the ability to germinate before the seeds reach its physiological maturity prior to desiccation tolerance acquisition (Bewley *et al*., 2013) though physiological maturity is often better as most seeds will have maximum desiccation tolerance. Understanding such underlying information may help in early harvesting of the species in order to avoid loss due to dehiscence.

Observation done in MARDI Pontian indicates that *C. liberica* seedling emergence from the soil occur at 90 days after sowing and shows about 60% of germination over a period of two months. The germination in coffee has been reported to be influenced by the maturity of the fruits as well as the method of processing which is believed to break the dormancy present in coffee seeds. Coffee seeds are usually harvested in bulk without proper selection of fruit maturity. This causes the seeds to perform poorly in the field with very slow and asynchronous germination. Harvesting at the right stage of maturity coupled with good drying techniques to suitable moisture content may result in improved performance of seeds for rootstock development. Identifying the right stage of development that has the highest viability is not only useful for propagation purposes but also useful for storage. Therefore, relationships between physiological aspects of the coffee seed, and its maturity phases and ripeness are vital. Subsequently, it is important to determine the right stage of harvesting maturity in terms of seeds germination and vigor. Identifying the right stage of development that has the highest viability is not only useful for the propagation purposes, but also useful for the storage. Owen (1957) stated that seeds with initially high viability can tolerate better towards unfavorable storage humidity and temperature than those of low initial viability. The principal factors influencing the viability of stored seeds are the moisture content and the temperature during the storage. In order for the seeds to maintain their viability when stored, the moisture content needs to be relatively low to minimize the metabolic activities. Therefore, the objectives of the study are:

1. To determine the suitable fruit maturity stages for seeds to be used in seedling establishment.
2. To determine the effect of various seed treatments in order to obtain a rapid germination rate.
3. To establish an appropriate drying and storage method for *C. liberica.*

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