UNIVERSITI PUTRA MALAYSIA

MATURITY STAGES, VARIATION WITHIN BUNCH AND DORMANCY BREAKING TREATMENTS IN RELATION TO GERMINATION OF OIL PALM (Elaeis guineensis Jacq.) D × P SEEDS

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FP 2021 13

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

October 2020

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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October 2020

Chairman : Professor Uma Rani Sinniah, PhD
Faculty : Agriculture

Oil palm is mainly propagated through dura × pisifera hybrid (D × P) seeds. In natural environment, oil palm seeds require more than eight months of storage to achieve 25% germination due to seed dormancy. Currently, commercial seed producers adopt heat treatment to alleviate dormancy in D × P seeds with monthly germination ranging from 62.2% to 72.0% within four months after being harvested. Since the demand for oil palm is increasing, there is a need to increase the supply of pre-germinated D × P seeds. Therefore, improvement in percentage of germination, uniformity, and reduction in time to germination will benefit the industry tremendously. This thesis focuses on both the pre- and post-harvest factors in improving the germination of oil palm D × P seeds. In the first experiment, the effect of harvesting at three maturity stages (18, 20 and 22 weeks after pollination or WAP) and six seed positions within a bunch (Proximal-Base, Proximal-Apex, Middle-Base, Middle-Apex, Distal-Apex and Distal-Base) on seed qualities were evaluated in a split-plot design. Seeds harvested at 20 WAP had 80% black coloured seeds, 19% moisture content with highest germination of 85.1%, compared with 70.5% and 80.9% for 18 and 22 WAP, respectively. Increasing seed maturity from 18 to 20 WAP showed more seeds shifting from semi-white and white to black (up to 40% more). Seeds located at the base region of the spikelets were smaller in size and were predominantly white with no differences in germination capacity. In the second experiment, the morphological and physiological performance of oil palm D × P seeds varying in colour were evaluated. Four replicates of 10 and 100 seeds, laid out in Completely Randomised Design (CRD) were used for seed characterisation and germination test, respectively. Thirty pre-germinated D × P seeds from each replicate were then transferred to the nursery for morphological, physiological and growth assessment. Germination test showed that all the seed types indicated similar germination percentage (more than 78%) and speed (13- 15 days of mean germination time). The nursery assessment showed that seedlings from the black seeds have higher growth (biomass, total leaf area, stem diameter) for the first 3 months due to the relatively larger size of the seed. No differences in growth were observed from 6 until 12 months after sowing. Physiological evaluation including net photosynthesis (5.30 to 18.13 µmol CO_2 m^{-2} s^{-1}), chlorophyll contents (5.70 to 9.68 mg cm^{-2}), stomatal conductance, transpiration rate as well as the intercellular CO_2 concentration showed different seed colour produces physiologically similar normal seedlings. The third experiment was designed to elucidate the effect of eight dormancy breaking methods (operculum removal, 60 days storage, 60 days heat treatment, 120 days storage, 60 days storage + heat treatment, 180 days storage, 120 days storage + heat treatment and control) on seed germination based on physical, morphological, and physiological dormancy characteristics. The imbibition test indicated that less than 7% mass increment was recorded in all treatments after 240 hours of imbibition, while the embryo showed 10 to 20% increase in moisture after imbibition. Final germination of more than 82% was obtained for heat treated seeds, and seeds stored prior to heat treatments, along with less than 13 days of mean germination time. Morphological dormancy evaluation indicated that the embryo was fully developed at 20 WAP, but application of heat treatment was able to accelerate the growth after being imbibed. The physiological dormancy aspect was found to be associated with reduction in peroxidase (POD) and catalase (CAT) activities, along with increase in α-amylase production in the endosperm and embryo tissues. The heat and storage treatments cause up to 36 % reduction in POD and 13% in CAT activity levels, with 9% (endosperm) and 26% (embryo) increment of α-amylase. Based on these results, it can be suggested that oil palm seeds exhibit non-deep physiological dormancy (PD), with heat treatment of 40 °C as the most effective and practical dormancy-breaking method for commercial seed production. In the fourth experiment, the efficacy of fluctuating temperature in comparison to constant 30 °C condition on oil palm D × P seed germination was assessed. D × P seed samples from similar sources were placed in two locations (Banting and Renggam), as a nested design. Oil palm seeds recorded higher germination percentages and speed at fluctuating temperature (70 to 85%) in comparison with the constant 30 °C (55 to 65%). Results indicated that higher temperature amplitude at Renggam showed 15% higher germination than at Banting. In addition, it was found that higher α-amylase activity (209.0 to 223.7 units/ ul) was observed for seed that was germinated under fluctuating temperature condition. From this study, harvesting oil palm D × P fruit bunch at 20 WAP, and application of heat-treatment (40 ±2 °C) prior to germination at ambient fluctuating temperature condition are recommended to obtain 85% final germination within 17 days.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERINGKAT KEMATANGAN, VARIASI TANDAN DAN RAWATAN PEMECAHAN DORMANSI TERHADAP PERCAMBAHAN BIJI BENIH D × P KELAPA SAWIT (*Elaeis guineensis* Jacq.)

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Kelapa sawit kebiasaannya ditanam melalui penggunaan biji benih hibrid dura × pisifera (D × P). Dalam keadaan semulajadi, biji benih kelapa sawit memerlukan lebih dari lapan bulan untuk mencapai 25% percambahan kerana isu dormansi biji benih. Ketika ini, pengeluar biji benih komersial menggunakan kaedah rawatan haba untuk mengatasi dormansi dalam biji benih D × P dengan purata 62.2% ke 72.0% percambahan dalam masa empat bulan selepas dituai. Oleh itu, peningkatan dalam peratusan percambahan, keseragaman dan pengurangan masa untuk bercambah akan memberi manfaat yang tinggi kepada industri. Tesis ini memberi fokus terhadap faktor sebelum dan selepas penuaian dalam meningkatan percambahan dalam biji benih D × P kelapa sawit. Dalam eksperimen pertama, kesan penuaian pada tiga peringkat kematangan (18, 20 dan 22 selepas didebungakan atau WAP) dan enam posisi biji benih dalam tandan (Proximal-Base, Proximal-Apex, Middle-Base, Middle-Apex, Distal-Apex dan Distal-Base) terhadap kualiti biji benih telah dikaji menggunakan reka bentuk belahan petak. Biji benih yang dituai pada 20 WAP mempunyai 80% biji benih berwarna hitam, 19% kandungan kelembapan bersama percambahan paling tinggi (85.1%), berbanding 70.5% dan 80.9% bagi 18 dan 22 WAP. Peningkatan kematangan biji benih dari 18 WAP kepada 20 WAP menunjukkan bahawa lebih banyak biji benih bertukar dari warna separa putih dan putih kepada warna hitam (lebih dari 40%). Biji benih yang terletak di bahagian bawah spikelet adalah bersaiz lebih kecil dan kebanyakannya berwarna putih, tanpa perbezaan dari segi percambahan. Dalam eksperimen kedua, prestasi morfologi dan fisiologi biji benih D × P yang berbeza warna telah dikaji. Empat replikasi bagi 10 dan 100 biji benih, menggunakan reka bentuk rawak lengkap (CRD) telah digunakan bagi karakter biji benih dan ujian

percambahan. Tiga puluh biji benih D × P pra-cambah dari setiap replikasi telah dipindahkan ke nurseri bagi penilaian morfologi, fisiologi dan pembesaran. Ujian percambahan menunjukkan bahawa semua jenis biji benih menunjukkan peratusan percambahan (lebih dari 78%) dan kelajuan percambahan (13-15 hari purata masa percambahan) yang sama. Penilaian di peringkat nurseri menunjukkan bahawa anak pokok dari biji benih berwarna hitam mempunyai pembesaran (biojisim, luas keseluruhan daun, diameter batang) yang lebih tinggi bagi 3 bulan pertama disebabkan saiz biji benih yang lebih besar. Tiada perbezaan dari segi pertumbuhan telah dilihat dari 6 ke 12 bulan selepas disemai. Kajian fisiologi yang merangkumi jumlah bersih fotosintesis (5.3 ke 18.13 µmol CO_2 m^{-2} s^{-1}), kandungan klorofil (5.7 ke 9.68 mg cm^{-2}), kekonduksian stomata, kadar transpirasi dan kepekatan CO_2 antara sel menunjukkan biji benih berbeza warna menghasilkan anak pokok yang sama prestasi dari aspek fisiologi. Eksperimen ketiga telah direka bagi menilai kesan lapan kaedah pemecahan dormansi (pembuangan operculum, penyimpanan selama 60 hari, rawatan haba selama 60 hari, penyimpanan 120 hari, penyimpanan 60 hari + rawatan haba, penyimpanan 180 hari, penyimpanan 120 hari + rawatan haba dan kawalan) terhadap percambahan biji benih berpandukan karakter dormansi fizikal, morfologi dan fisiologi biji benih. Ujian rendaman menunjukkan bahawa kurang dari 7% peningkatan jisim telah ditunjukkan bagi semua kaedah rawatan selepas 240 jam rendaman, manakala embrio menunjukkan 10 ke 20% peningkatan dalam kelembapan selepas rendaman. Percambahan akhir berjumlah lebih dari 82% telah direkodkan bagi biji benih yang menggunakan rawatan haba, dan disimpan sebelum rawatan haba, di samping mempunyai kurang dari 13 hari tempoh purata percambahan. Penilaian dormansi morfologikal menunjukkan bahawa embrio telah matang sepenuhnya pada 20 WAP, akan tetapi penggunaan rawatan haba telah berjaya mempercepatkan pertumbuhan selepas rendaman. Penggunaan rawatan haba dan penyimpanan sebelum rawatan haba menyebabkan penurunan 36% bagi POD dan 13 % bagi aktiviti CAT, di samping 9% peningkatan α-amylase di endosperma dan 26% di embrio. Hasil kajian menunjukkan bahawa biji benih kelapa sawit mempunyai non-deep physiological dormancy (PD), dan penggunaan rawatan haba 40 °C sebagai kaedah paling efektif dan praktikal bagi pengeluar biji benih komersial. Dalam eksperimen keempat, keberkesanan percambahan di suhu turun naik berbanding suhu sekata (30 °C) bagi biji benih kelapa sawit telah dinilai. Sampel biji benih D × P dari sumber yang sama telah diletakkan di dua lokasi (Banting dan Renggam), secara reka bentuk tersarang. Biji benih kelapa sawit merekodkan peratusan percambahan dan kelajuan yang lebih tinggi di suhu turun nauk (70 ke 85%), berbanding dengan suhu sekata 30°C (55 ke 65%). Keputusan menunjukkan bahawa perbezaan suhu yang lebih tinggi di Renggam menyebabkan pertambahan percambahan sebanyak 15% berbanding di Banting. Tambahan pula, aktiviti α-amylase (209.0 ke 223.7 units/ ul) yang lebih tinggi telah dilihat bagi biji benih yang bercambah di suhu turun naik. Dari kajian ini, penuaian tandan kelapa sawit D × P pada 20 WAP, dan penggunaan rawatan haba (40 ±2 °C) sebelum percamban di suhu turun naik adalah dicadangkan bagi mencapai 85% percambahan akhir dalam masa 17 hari.

ACKNOWLEDGEMENTS

I would like to express my highest appreciation to the chairman of my supervisory committee, Prof. Dr. Uma Rani Sinniah for her guidance and support throughout my studies. Her unwavering enthusiasm in academia and research inspired me to be passionate with my work, while her kind advice always managed to get me back on the right track. Being under her mentorship had made my PhD journey more than a wonderful experience. I would also like to convey my sincere gratitude to the supervisory committee members, Prof. Dr. Adam bin Puteh, Assoc. Prof. Dr Parameswari Namasivayam, and Dr David Ross Appleton for their valuable suggestions and comments which tremendously helped me in completing my dissertation.

I would like to thank Ministry of Higher Education Malaysia and Universiti Putra Malaysia for the financial support through SLAB scholarship and Junior Academic Scheme during this study period.

My gratitude is also extending to Sime Darby Research Sdn Bhd. particularly to Mr Mohaimi Mohamed, Mr Aminuddin and Mr Fazly for providing the materials, facilities, and industrial insights throughout this study. Not forgetting, to all the lecturers and staffs at Department of Crop Science, Faculty of Agriculture, UPM, for their kind assistance.

Special thanks to my fellow graduate friends at the seed technology and cryopreservation laboratories for the wonderful moments we shared together. Good friends are indeed hard to find, harder to leave, and impossible to forget.

Finally, I owe an enormous debt of gratitude to my parents, brother, and sisters for their continuous encouragement. To my wife and son, thank you for being my constant source of strength and joy. I would not have been able to complete this dissertation without their unconditional support and love.

Thank you.

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

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ABA	Abscisic acid
ANOVA	Analysis of variance
CAT	Catalase
CRD	Completely Randomized Design
D × P	Dura × Pisifera
DAI	Days after imbibition
df	Degree of freedom
DW	Dry weight
E:S	Embryo: seed
FGP	Final germination percentage
GA	Gibberellic acid
ISTA	International Seed Testing Association
LSD	Least Significant Differences
MD	Morphological dormancy
MGT	Mean germination time
MPD	Morphophysiological dormancy
MPOB	Malaysian Palm Oil Board
PD	Physiological dormancy
PM	Physiological maturity
POD	Peroxidase
PY	Physical dormancy
p-value	probability value

RCBD	Randomised Complete Block Design
rpm	revolutions per minute
SAS	Statistical Analysis Software
SDSAS	Sime Darby Seeds and Agricultural Services
spp.	species
SPU	Seed production unit
USDA	United States Department of Agriculture
WAP	Weeks after pollination

CHAPTER 1

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is known as the highest yielding oilseed in the world. On average, 4.0 metric tonnes of oil are produced per hectare of land every year, far exceeding the yield of other sources of oilseed such as soybean, sunflower and rapeseed (Malaysian Palm Oil Council, 2018). According to the USDA (2020), total world vegetable oil consumption in 2018/2019 was 204.83 million metric tonnes with 74.62 million metric tonnes (36.4%) being palm oil, followed by soybean (27.8%), rapeseed (13.5%) and sunflower seed (9.3%). It was estimated that oilseed consumption will exceed 240 million metric tonnes in 2050, based on current requirement along with population growth forecast (Corley, 2009). The increasing demand for oilseed supply is a challenge for the oil palm industry to be more efficient particularly in ensuring higher fresh fruit bunch (FFB) yield. To achieve this, both genotypic and environmental aspect are crucial, along with incorporation of efficient management practise.

The main method of oil palm propagation is through seeds, using the Dura × Pisifera (D × P) hybrid seeds, developed through breeding programmes. It was reported that the production for Malaysian D × P seed production had declined from 88 million seeds in 2008 (Kushairi et al., 2010) to less than 60 million seeds per year in 2019 (Malaysian Palm Oil Board, 2020), despite the increasing forecasted demand of planting materials for 2050 (Corley, 2009). In addition, oil palm replanting programs are extensively conducted in recent years to replace fields that have exceeded the economic period of 25 years. Therefore, continuous supplies of pre-germinated seeds are needed in oil palm nurseries and estates. Currently, the production of D × P pre-germinated seeds are based on a standard guideline described in Malaysian Standard MS 157: 2005 Oil Palm Seeds for Commercial Planting- Specification (Department of Standards Malaysia, 2005). Seeds produced using the above guidelines brings high price which range from RM 2.35 to RM 3.80 per pre-germinated seed.

Oil palm seeds are known to have exceptionally low and inconsistent germination in natural environments as they are highly dormant. It was reported that D × P seeds recorded less than 25% germination after eight months of storage (Norsazwan et al., 2016). Hussey (1958) previously suggested that the seed was physiologically dormant with hard endocarp which prevents optimal absorption of oxygen into the embryo. He was also the first to propose the idea of using heat treatment of 38 to 40 ºC to alleviate dormancy in *tenera* seeds. Since then, a majority of the studies conducted in relation to dormancy-breaking and germination in oil palm seeds revolved around the usage of heat-treatment (Rees, 1962; Mok, 1982; Martine et al. 2009; Fondom et al., 2010; Green et al., 2013). Findings from these studies were used as a basis of current adoption of heat treatment of 40 ± 2 °C for a period of 40 to 60 days prior to germination by most commercial seed producers in Malaysia (Corley and Tinker, 2015; Rao and Choong, 2014; SDSAS SOP, 2020). While heat treatment was indeed found to be the most effective dormancy-breaking method for oil palm D × P seed by far, the resulting germination was still inconsistent. Commercial D × P seed production recorded an average of monthly germination ranging from 62.2% to 72% (Sime Darby Seeds and Agricultural Services, Seed Production Unit Data) using the above-mentioned method. Sporadic germination pattern was observed throughout the standard 60 days germination evaluation period. Theoretically, if seed dormancy had been successfully alleviated, the seeds should be able to germinate uniformly with higher average germination percentage (Copeland and McDonald, 2001; Baskin and Baskin, 2004).

To address the germination issue in oil palm seed, it is important to evaluate the seed from pre- and post-harvest perspective. Pre-harvest encompasses the seed development phase upon successful pollination and fertilisation. During developmental process, the seed will undergo a series of morphological and physiological changes which will eventually influence the quality of the harvested seeds (Baskin and Baskin, 2004). In general, harvested fruit bunch consisted of approximately 1000 to 3000 seeds, with high phenotypic variability including seed colours, sizes, and oil content (Corley and Tinker, 2015). This variation may be attributed to differences in the genotypes as well as the environmental effect during the development process. The management practise could also contribute to this variation particularly with regards to the standard operating protocol used, including harvest time, and segregating the seeds according to specific criteria such as colour or sizes. In oil palm seed production, seed colour is another interesting aspect which causes huge commercial implication. During seed processing, white coloured seeds will be separated and discarded (SDSAS SOP, 2020), as the consumer generally favours black coloured pre-germinated seeds. Currently, the Standard Operation Procedure (SOP) in which the white seeds were discarded is solely based on consumer demand of black seed, influenced by their perception on the external appearance of the seeds without any scientific basis to support it.

On the other hand, post-harvest refers to handling after harvest and the application of specific dormancy-breaking treatments prior to germination. Seeds that confer primary dormancy upon successful seed developmental phase will require additional dormancy-breaking measures. According to Baskin and Baskin (2014), most palm (Arecaceae) family exhibit morphophysiological dormancy. This means, seeds are generally able to imbibe water, however, remain dormant due to underdeveloped embryo and require long germination time to alleviate the physiological dormancy component. As mentioned previously, current seed producers adopted heat treatment of 40 ± 2 °C for 60 days as a method to alleviate seed dormancy in oil palm based on findings from previous studies (Rees, 1962; Mok, 1982; Martine et al., 2009; Fondom et al., 2010; Green et al., 2013). Although heat treatment application was able to accelerate oil palm seed germination, the germination percentage and speed were inconsistent in commercial seed production. In theory, higher germination percentage and speed could be attained once the seed dormancy had been successfully alleviated. Seed dormancy encompasses three major components including physical, morphological, and physiological dormancy, which relates to each specific dormancy-breaking mechanism (Baskin and Baskin, 2004). Understanding the effect of different treatments towards these components could identify the best and most practical treatments for germination improvement. Therefore, there is a need to systematically evaluate the efficacy of different dormancy-breaking treatments according to the physical, morphological, and physiological dormancy attributes of oil palm seeds.

In addition to identification of an efficient dormancy breaking method, the germination condition is also crucial for optimum germination. Physical factors such as the temperature, water availability, and atmospheric condition are known to highly influence seed germination (Copeland and McDonald, 2001). However, among these factors, germination temperature was found to be the most crucial component; since slight differences in temperature may results in huge implication on endogenous moisture level, hormone production, and enzyme activity (Roberts, 1988; Vleeshouwers et al., 1995; Probert, 2000; Milbau et al., 2009). In oil palm seed production, commercial seed producers adopted constant temperature of 30 ± 2 °C at their germination facilities (Corley and Tinker, 2015; Rao and Choong, 2014). In general, studies have shown that majority seed species germinated better in a temperature regime depicting its habitat or origin. For this reason, some seeds are known to require diurnal temperature fluctuations to ensure survival of the emerged seedlings (Ellery, 2002; Brandel and Jensen, 2005; Vandelook and Van Assche, 2008; Liu et al., 2013). Therefore, it is possible that the current constant temperature used by the industry might not be optimal for oil palm seed germination, resulting in non-uniform germination over a period of 60 days. To further improve germination capacity, studies on endogenous hormonal balance should be attempted through direct or indirect detection method. α- amylase enzyme is one of the commonly associated enzymes with germination capacity which influence the balance of GA and ABA hormones (Kanzaki et al., 1993; Joshi, 2018).

The overall perspective of this study was to improve seed quality in oil palm by understanding both the pre- and post-harvest processes to improve germination and obtain rapid and synchronous germination. Therefore, the objectives of this study include:

i. To evaluate the effect of seed maturity stages and position of fruitlets on the bunch on seed quality

ii. To evaluate morphological and physiological performance of oil palm D × P seeds varying in colour from germination until nursery stage
iii. To elucidate the effect of selected dormancy breaking methods on seed germination based on physical, morphological, and physiological dormancy characteristics and
iv. To compare the efficacy of fluctuating temperature condition against the current constant temperature on germination performance

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BIODATA OF STUDENT

Mohd Norsazwan bin Ghazali was born on 6th August 1989 in Teluk Intan, Perak. He received his early education in his hometown at Sekolah Kebangsaan Kampung Bahagia and Sekolah Menengah Sains Teluk Intan. He then pursued Australian Matriculation program, before continuing his bachelor's degree in Science (Agriculture) at University of Western Australia (2009 - 2012). In 2013, Norsazwan was appointed as Cadet Assistant Research Officer in Agronomy for oil palm and coconut at United Plantations Berhad. After a year of industrial training, Norsazwan commenced his Master of Science (Seed Science and Technology) at Universiti Putra Malaysia (UPM). The experience he gained from his MSc had motivated him to pursue his PhD in Plant Science at UPM, from 2017 until 2020.

LIST OF PUBLICATIONS

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