UNIVERSITI PUTRA MALAYSIA

CLONAL PROPAGATION AMENABILITY OF SELECTED ELITE (DxP) AND CLONAL TENERA ORTETS OF OIL PALM (Elaeis guineensis Jacq.)

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By

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

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

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November 2019

Chairman : Associate Professor Mohd. Puad Abdullah, PhD
Faculty : Biotechnology and Biomolecular Sciences

The scale up of high quality oil palm ramets production is a main priority for all commercial tissue culture laboratories. To date, oil palm cloning technology is generally inefficient with low embryogenesis rate, genotype dependency and insufficient elite ortets. As the trends in culture development for mass propagation remains unpredictable, a thorough investigation of the relevant process is immensely important. This study aims to evaluate and expound the performance of selected elite *tenera* ortets for large scale production specifically during the stages of callogenesis, embryogenesis and embryoids maintenance en route for enhanced oil palm yield. Leaf explants were cultured on Murashige and Skoog (1962) medium with the current standard production protocol. Clonal ortets yielded callus of 1,340 ± 149 lines significantly ($F=7.46$, $P=0.014$) which refers to the frequency of callus initiated from the edge of cultured immature leaflet explants at 0.52-fold higher compared to elite *tenera* (DxP) ortets at 698 ± 182 lines. Besides, clonal ortets demonstrated shorter callus induction time than elite *tenera* ortets. The earliest callus formation for clonal and elite *tenera* ortets were observed after 93.90 ± 4.27 days and 107.00 ± 7.11 days respectively ($F=2.73$, $df=1, 18$; $P>0.05$). Embryoid lines achieved were significantly higher ($F=38.00$, $P=0.00$) in clonal ortets (794 ± 108 lines) by 8.6-fold against elite *tenera* ortets (91.6 ± 35.0 lines). In addition to that, differences of the embryoid line production rapidity between ortet types were found to be statistically significant ($F=5.71$, $df=1, 18$: $P<0.05$) in which clonal ortets developed embryoids faster by an average of 68 days earlier than the elite *tenera*. Statistical analysis of the data showed that there was positive correlation ($r=0.69449$, $P<0.05$) between the total of embryoid lines formed with the total production of callus derived from elite *tenera* and clonal ortets throughout the production process. The embryoid lines are represented as the frequency of embryoid developed from the callus which were observed as whitish in colour and appear with smooth surface. This indicates that for both types of ortet, the initiation of embryoid is proportionate to the production of callus lines ($r=0.69449$, $P<0.05$). The growth models for describing polyembryoids (PE) development for clonal and elite *tenera* ortets versus time of subculture or pattern were established. Meanwhile, the growth rate pattern of elite *tenera* ortets appeared to be non-uniformed,

or uncertainty in the culture growth development compared to the series of clonal ortets tested. The potential of ortets development therefore could be estimated from a logistic growth curve fitted through the PE culture growth (in weight, g) against the duration of week after the initial culture. The growth patterns of clonal ortets are observed to be more predictive which facilitate further growth prediction. Therefore, the clonal ortets offers the best source for oil palm propagation optimization towards upscaling the ramets production in commercial scales.

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

KEBOLEH-TERIMAAN PEMBIAKAN KLON PADA ORTET ELIT (DxP) DAN KLON TENERA SAWIT (*Elaeis guineensis* Jacq.) TERPILIH

Oleh

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Penghasilan ramet sawit berskala besar dan berkualiti menjadi keutamaan bagi setiap makmal kultur tisu komersial. Sehingga kini, proses pengklonan sawit secara amnya adalah tidak cekap, dengan kadar embriogenesis yang rendah, kebergantungan kepada genotip dan kekurangan sumber ortet terpilih. Memandangkan corak perkembangan bahan kultur ke arah penghasilan ramet sawit berskala besar masih tidak dapat dijangka, adalah amat penting untuk kajian terperinci dilakukan. Kajian ini bertujuan untuk menilai dan mendapatkan pemahaman terhadap prestasi ortet untuk pengeluaran berskala besar dengan penumpuan kajian pada peringkat pembentukan kalus, embrioid dan multiplikasi embrioid ke arah meningkatkan hasil minyak sawit. Eksplan daun telah dikultur ke dalam medium Murashige and Skoog (1962) menggunakan protokol pengeluaran piawai sedia ada. Ortet klonal secara signifikannya ($F=7.46$, $P=0.014$) menghasilkan bilangan kalus lebih tinggi sebanyak 0.52-ganda iaitu 1,340 ± 149 baris berbanding ortet *tenera* elit (DxP) iaitu 698 ± 182 baris. Baris merujuk kepada kekerapan kalus terbentuk daripada pinggir eksplan daun muda yang dikultur. Selain itu, ortet klonal juga menunjukkan tempoh penghasilan kalus yang lebih pendek dengan penghasilan kalus terawal bagi ortet klonal adalah selepas 93.90 ± 4.27 hari dan ortet *tenera* elit pula hanya selepas 107.00 ± 7.11 hari ($F=2.73$, $df=1, 18$; $P>0.05$). Pencapaian pembentukan embrioid juga lebih tinggi secara signifikan ($F=38.00$, $P=0.00$) bagi ortet klonal (794 ± 108 baris), iaitu 8.6-ganda berbanding ortet *tenera* elit (91.6 ± 35.0). Malah, perbezaan tempoh penghasilan embrioid di antara kedua-dua jenis ortet juga adalah signifikan ($F=5.71$, $df=1, 18$: $P<0.05$), iaitu ortet klonal dapat menghasilkan embrioid seawal 68 hari berbanding ortet *tenera* elit. Analisis statistik menunjukkan terdapat korelasi secara positif ($r=0.69449$, $P<0.05$) di antara bilangan embrioid yang dihasilkan, yang diwakili oleh struktur berwarna putih dan mempunyai permukaan yang licin serta terbentuk daripada kalus, berbanding dengan jumlah kalus dihasilkan daripada ortet elit *tenera* dan ortet klonal ketika proses pengeluaran. Ini menunjukkan bahawa pembentukan embrioid adalah berkadar langsung dengan pengeluaran kalus bagi kedua-dua jenis ortet ($r=0.69449$, $P<0.05$). Model pertumbuhan perkembangan poliembrioid untuk ortet tenera elit dan klonal terhadap masa subkultur atau corak pertumbuhan telah dibangunkan. Sementara itu, corak pertubuhan bagi ortet

tenera elit didapati tidak seragam berbanding ortet klonal yang dibuktikan lebih sekata. Justeru, potensi pertumbuhan ortet dapat dijangka berdasarkan lengkung pertumbuhan logistik dari pertumbuhan kultur PE (berat, g) dan tempoh kultur PE daripada permulaan kultur. Corak pertumbuhan ortet klonal didapati lebih dapat diramal yang memudahkan jangkaan pertumbuhan pada masa seterusnya. Justeru, ortet klonal adalah sumber terbaik bagi mengoptimumkan pembiakan ramet sawit ke arah meningkatkan pengeluaran ramet berskala komersial.

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'My Lord, increase me in knowledge..!'

Surah Taha (20: 114)

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This thesis 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

ANOVA	Analysis of Variance
cm	centimetre
g	gram
ha	hectare
m	meter
mm	milimeter
kg	kilogram
t	tonne
r	correlation coefficient
R^2	coefficient of determination
%	percentage
0ºC	degree celcius
2,4-D	2,4-dichlorophenoxyacetic acid
MS	Murashige and Skoog media
FFB	Fresh fruit bunches
CPO	Crude palm oil
PK	Palm kernel
MPOB	Malaysian Palm Oil Board
PGRs	Plant growth regulator
TCDS	Tissue culture database system
SpID	Sampling identification
OTB	oil to bunch

CHAPTER 1

INTRODUCTION

The golden crop of Malaysia, the oil palm was originally introduced to Malaysia in 1870 from Ghana. The cultivation began in the last century by planting the *dura* fruit type in the plantation. However, the yield was marginal (Kushairi and Rajanaidu, 2000). The first wave of yield improvement of up to 30% was by switching from *dura* to the *tenera* (DxP) planting materials (Beirnaert and Vanderweyen, 1941). Since then, oil palm breeders have continuously devoted great efforts in developing traits for genetic improvement program (Soh et al., 2009). The genetic potential of the palm has been reported to be 18.2 tonnes of oil/hectare/year (t/ha/yr) (Corley, 1998; Chan et al., 2000) and cloning such palm would provide high-yielding planting materials for the industry, which would be the second wave in yield improvement. In 2018, the national average annual oil yield has been stagnating at 3.42 tonnes of oil/ha/yr with the fresh fruit bunches (FFB) achievement of 17.9 t/ha/yr. Most of the current oil palm planting materials are from the high yielding materials which are propagated through cross-pollination of selected *dura* x *pisifera* (DxP) seed materials with improved genetic potential. Generally, conventional breeding methodology for superior traits is time consuming and often produces a plenty of variation.

Reports on success in oil palm cloning was first announced in the mid-seventies (Jones, 1974; Rabechault and Martin, 1976) and a little later in the early eighties in Malaysia (Wooi et al., 1981; Paranjothy and Othman, 1982).

Oil palm possesses a single growing point and due to its feature that does not have suckers or bud grafting, oil palm ramets cannot be vegetatively multiplied through common technique as applied to other plants e.g. roses, bamboo and rattans (Corley and Tinker, 2016). Thus, cloning via plant tissue culture techniques is the only method to produce and propagate uniform planting materials.

Soh (2018) described the selection of outstanding ortets based on oil to bunch is preferrable due to higher heritability against fresh fruit bunches (FFB). Inefficient oil palm tissue culture process, incidence of mantling and high cost of ramets are also the current prevailing drawbacks. The ortet source limitation has led the tissue culturist to investigate and assess the potential and performance of clonal and elite *tenera* (DxP) ortets in the cloning process for mass propagation. Clonal ortet refers to the selected planted palms derived from tissue culture process that meets the requirement of Malaysian Standard MS 2099: 2008 - Oil palm clones for commercial planting – Specification for ortet selection. The inclination of utilizing clonal ortets in commercial tissue culture laboratory for mass propagation has been recently documented (Roowi et al., 2010; Tarmizi et al., 2017; Febrianti, 2018; Choo et al., 2018; Ong-Abdullah et al., 2018; Soh, 2018). Since information on the oil palm tissue culture protocols are scarce, thorough investigation on the culture development from the initial stages of callogenesis, embryogenesis and polyembryoids maintenance are needed to optimize the ortets potential.

Therefore, this study was undertaken with a prime goal to improve the efficiency of the oil palm cloning process with the following specific objectives.
The objectives of this study are:

1. to differentiate between the callogenesis and embryogenesis potentials of elite *tenera* and clonal ortets.
2. to establish the growth patterns of polyembryoids derived from elite *tenera* and clonal ortets

This work will advance the current knowledge on oil palm cloning, specifically on ortets performances at the stage of callogenesis, embryogenesis and polyembryoid maintenance that eventually will contribute further to the development of a growth model which will eventually provide efficient information for oil palm ramets projection, particularly for commercial scale propagation.

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