



**UNIVERSITI PUTRA MALAYSIA**

***ESTABLISHING QUALITY CONTROL OF OIL PALM SUSPENSION  
CULTURE VIA FLOW CYTOMETRY, IMAGE CYTOMETRY AND  
FLUORESCENCE *in situ* HYBRIDIZATION***

**MOHD RAZIK BIN MIDIN**

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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 Master of Science**

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**Faculty: Science**

Oil palm industry targets to provide high quantities of clonal palms with agronomic trait *via* clonal propagation of elite palms. Clonal propagation of oil palm through tissue culture process is achieved *via* callus or suspension cultures. The occurrence of *in vitro* culture stress may generate genome variability such as genome size and ploidy level alteration. Hence, several cytological tools were utilized in this study to observe any changes that might occur in adult clonal palms, their respective suspension cultures and regenerant plantlets. The tools used were flow cytometry (FCM), 18S-25S ribosomal DNA-fluorescence *in situ* hybridization (rDNA-FISH), and image cytometry (ICM). The findings of FCM on the nuclear genome sizes of the four adult clonal palms (using leaf samples from Frond-1) varied from  $2.59 \pm 0.19$  pg to  $2.91 \pm 0.14$  pg while for 8-months-old regenerant plantlets (five replicates for each samples) varied from  $2.14 \pm 0.21$  pg to  $3.05 \pm 0.11$  pg. Adult clonal samples and their respective regenerants showed the same ploidy level which is diploid, indicated

by FCM analysis. Oil palm suspension culture materials were limited, hence produced less than 1,000 nuclei and were unable to be analysed by FCM. This led to the utilization of rDNA-FISH and development of ICM technique which used the application available in the PAX-it image analysis software (Midwest Information Systems, USA). Based on the rDNA-FISH analysis on the suspension culture and regenerant plantlet materials, two hybridization signals were observed on the interphase nuclei indicating diploid ploidy level. ICM analysis of the suspension cultures revealed that the nuclear genome size ranged from 4.71 pg to 5.49 pg while the integrated optical density (IOD) values for all suspension cultures ranged from 0.06-0.18 arbitrary unit (a.u.). The pattern showed that the cell cycle of suspension cultures was lagging during the G1-S-G2 phase, hence explaining the slow proliferation rate. In summary, it was observed that diploid ploidy state was maintained throughout the adult clonal palms, their suspension cultures and regenerant plantlets while the genome size of suspension culture materials were higher than their adult clonal palms and regenerants. This study concluded that the quality control of oil palm suspension cultures could be established by the above-mentioned tools based on the estimated nuclear genome size and ploidy level. ICM tool developed in this study can also be applied to analyze the friable suspension calli cultures which are the starting material for suspension cultures. This would ensure the oil palm's clonal fidelity and improve the efficiency and robustness of suspension cultures.

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**KAWALAN KUALITI KULTUR AMPAIAN SAWIT MELALUI KAEDAH SITOMETRI ALIRAN, SITOMETRI IMEJ DAN PENGHIBRIDAN *in situ* BERPENDARFLUOR**

Oleh

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**Januari 2013**

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Sasaran industri sawit adalah untuk membekalkan kuantiti sawit berciri agronomi pada kadar kuantiti yang tinggi melalui propagasi sawit elit. Propagasi sawit melalui teknik kultur tisu dicapai melalui kalus atau kultur ampaiian sawit. Tekanan *in vitro* yang berlaku berkemungkinan menghasilkan variasi pada genom, seperti saiz genom dan aras ploidi. Justeru itu, beberapa kaedah sitologi telah digunakan dalam kajian ini bagi memerhati sebarang perubahan yang mungkin berlaku pada klon dewasa sawit, kultur ampaiian dan juga planlet regeneran. Kaedah-kaedah tersebut adalah sitometri aliran (FCM), 18S-25S ribosomal DNA-penghibridan *in situ* berpendarfluor (rDNA-FISH) dan sitometri imej (ICM). Hasil analisis FCM terhadap saiz genom klon sawit dewasa (menggunakan pelepah -1) adalah di antara  $2.59 \pm 0.19$  pg hingga  $2.91 \pm 0.14$  pg manakala saiz genom bagi planlet regenerannya yang berusia 8 bulan (5 replikasi bagi setiap sampel) berjulat diantara  $2.14 \pm 0.21$  pg sehingga  $3.05 \pm 0.11$  pg. Klon sawit dewasa dan planlet regenerannya menunjukkan aras ploidi yang sama

iaitu diploid, ditentukan melalui analisis FCM. Material kultur ampaiian sawit adalah terhad, justeru itu menghasilkan populasi nukleus kurang dari 1,000 menyebabkan ia tidak boleh dianalisis melalui FCM. Ini telah membawa kepada penggunaan rDNA-FISH dan pembangunan teknik ICM yang menggunakan aplikasi dalam perisian analisis imej PAX-it (Midwest Information Systems, USA). Berdasarkan kaedah rDNA-FISH pada kultur ampaiian sawit dan planlet regeneran, dua isyarat hibridisasi di atas nukleus interfasa dapat diperhati menandakan aras ploidi diploid. Analisis ICM terhadap kultur ampaiian sawit menunjukkan saiz genomnya berjulat di antara 4.71 sehingga 5.49 pg manakala puncak ketumpatan optik bersepadu (IOD) berjulat di antara 0.06-0.18 arbitrari unit (a.u.). Corak ini menunjukkan perkembangan kitar sel kultur ampaiian adalah perlahan pada fasa G1-S-G2, seterusnya menerangkan kadar proliferasi yang perlahan. Keseluruhannya, dapat diperhati bahawa aras diploid dikekalkan sepanjang klon sawit dewasa, kultur ampaiian dan planlet regeneran manakala saiz genom kultur ampaiian adalah lebih tinggi berbanding klon sawit dewasa dan planlet regenerannya. Kesimpulannya, kualiti kultur ampaiian boleh ditentukan melalui kaedah sitologi yang digunakan dengan merujuk pada saiz genom dan aras ploidi. Kaedah ICM yang dibangunkan dalam kajian ini juga boleh diaplikasi untuk menganalisis kalus 'friable' yang digunakan sebagai bahan pemula untuk kultur ampaiian. Ini akan memastikan ketulenan klon sawit dan meningkatkan keberkesanan kultur ampaiian.

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