



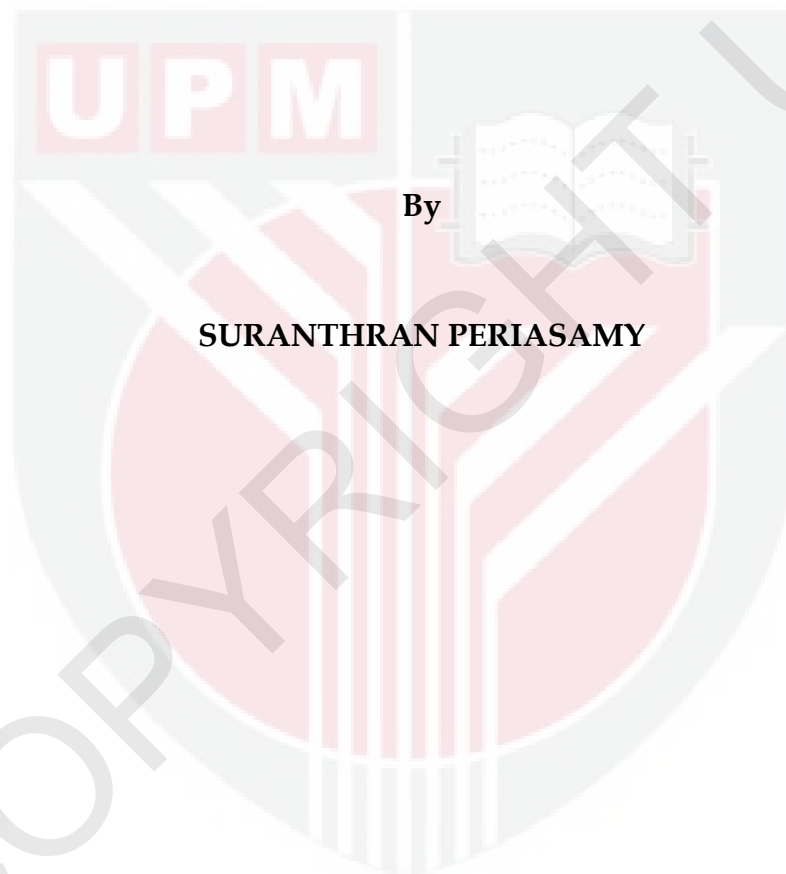
**UNIVERSITI PUTRA MALAYSIA**

***CRYOPRESERVATION OF ZYGOTIC AND SOMATIC EMBRYOS OF OIL PALM  
(*Elaeis guineensis* Jacq.) FOR GERMPLASM CONSERVATION***

**SURANTHRAN PERIASAMY**

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**CRYOPRESERVATION OF ZYGOTIC AND SOMATIC EMBRYOS OF  
OIL PALM (*Elaeis guineensis* Jacq.) FOR GERMPLASM  
CONSERVATION**



By

**SURANTHRAN PERIASAMY**

**Thesis Submitted to the School of Graduate Studies,  
Universiti Putra Malaysia, in Fulfilment of the Requirements  
for the Degree of Master of Science  
April 2012**

## DEDICATION

This manuscript is specially dedicated to my supreme almighty  
'SIVAPERUMAN'.



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

**CRYOPRESERVATION OF ZYGOTIC AND SOMATIC EMBRYOS OF  
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**SURANTHRAN PERIASAMY**

April 2012

**Chair : Associate Professor Uma Rani Sinniah, PhD**

**Faculty : Agriculture**

The objective of this research is to establish of refinement protocol on cryopreservation of zygotic embryos (ZE) (direct desiccation) and somatic embryos (SE) (vitification techniques) of oil palm (*Elaeis guineensis* Jacq.). To support the growth and development of oil palm ZE, initially, the effect of plant growth regulators (PGR) and activated charcoal (AC) on *in vitro* regeneration and seedlings development from oil palm (varieties Dura and Tenera) ZE was assessed. ZE were cultured on Murashige and Skoog (MS) medium supplemented with a blend of 0.05 or 0.1 mgL<sup>-1</sup> of each PGR (gibberellic acid, 6-benzlaminopurine and α-naphthaleneacetic acid) with or without 2 gL<sup>-1</sup> AC. Growth and development of the embryos were affected by the type of medium. ZEs cultured on MS medium supplemented with both PGR and AC enhanced shoot initiation and subsequent plantlet development, while PGR supplemented MS media without AC led to

abnormal growth suggesting that AC is indispensable for oil palm *in vitro* seedlings regeneration. The best medium for growth and development of plantlets was MS medium supplemented with 0.1 mgL<sup>-1</sup> PGR and 2 gL<sup>-1</sup> AC which showed significant variation compared to the remaining media formulations. After that, the effects of desiccation and freezing on survival of oil palm ZE, were assessed using the above mentioned media. ZE of variety Dura and hybrid Tenera were subjected to desiccation for 8 h. ZE were analysed for free water content (WC) and resurgence ability after each desiccation and freezing period. The survival was at its maximum (Dura 80%) and (Tenera 70%) when the desiccated ZE containing ~0.14 gH<sub>2</sub>O g<sup>-1</sup> fw of WC and below in which abnormal or no survival was recorded. This optimal WC not only assisted oil palm ZE to sustain their cellular integrity but also retained their regeneration potential following cryopreservation. On the contrary, ZE with WC above 0.24 gH<sub>2</sub>O g<sup>-1</sup> dw or below 0.16 gH<sub>2</sub>O g<sup>-1</sup> dw lost their viability as well as their cellular integrity attributable to either excess WC or excessive loss of free water, after desiccation and successive freezing. Scanning electron microscopic observations confirmed that there was no noteworthy distinction in morphology of epidermal layer after desiccation and successive freezing. Thus for successful cryopreservation of oil palm ZE, they should be desiccated to a WC of 0.14 gH<sub>2</sub>O g<sup>-1</sup> fw and 80% survival can be obtained. In the experiment concerning cryopreservation of oil palm clonal material using polyembryoid, the effect of various loading solutions (LS) and vitrification solutions (VS) and their time of exposure on

survival of polyembryoid in liquid nitrogen (LN) was evaluated. *In vitro* grown polyembryoid of oil palm were successfully cryopreserved by vitrification with 45% survival. Individual polyembryoid, which were separated and excised from two-month old (from polyembryoid initiation) culture clumps, were precultured in liquid MS medium supplemented with 0.5 M sucrose for 12 h and then treated with a mixture of 10% (w/v) DMSO plus 0.7 M sucrose for 30 min all at  $26 \pm 2^\circ\text{C}$ . Osmo-protected polyembryoid were first treated with VS (PVS2 - 30% (w/v) glycerol plus 15% (w/v) EG plus 15% (w/v) DMSO plus 0.4 M sucrose) for 5 min at  $26 \pm 2^\circ\text{C}$  and plunged directly into LN. Following rapid warming in a water-bath at  $38 \pm 2^\circ\text{C}$  for 90 sec, the polyembryoid were washed for 20 min at  $26 \pm 2^\circ\text{C}$  with liquid MS medium containing 1.2 M sucrose. They were then transferred onto solid MS medium 3% (w/v) sucrose and 0.75% (w/v) agar. The polyembryoid were kept in the dark for seven days prior to exposure to light (16 h photoperiod cycle). Direct shoot initiation was observed approximately after three weeks after culture. In overall, best culture medium for ZE growth is MS with  $0.1 \text{ mgL}^{-1}$  PGR and  $2 \text{ gL}^{-1}$  AC and approximately  $0.14 \text{ gH}_2\text{O g}^{-1}$  fw of WC shows highest survival in LN treatment for both Dura and Tenera; 12 h 0.5 M sucrose precultured polyembryoid treated with L5, 30 min and PVS2, 5 min was successfully cryopreserved in this study.

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

**PENKRIOWETAN EMBRIO ZIGOTIK DAN SOMATIK  
KELAPA SAWIT (*Elaeis guineensis* Jacq.) UNTUK  
PEMULIHARAAN GERMPLASMA**

Oleh

**SURANTHRAN PERIASAMY**

April 2012

**Pengerusi : Profesor Madya Uma Rani Sinniah, PhD**

**Fakulti : Pertanian**

Objektif kajian ini ialah pertubuhan terperinci protokol krioawetan embrio zigotic (pengeringan udara) dan embrio somatik (teknik pemvitreusan) kelapa sawit (*Elaeis guineensis* Jacq.). Pada mulanya, untuk menyokong pertumbuhan dan perkembangan embrio zigotik kelapa sawit, kesan penggunaan pengawalatur tumbuhan (PGR) dan arang teraktif (AC) telah dinilai di dalam penjanaan semula 'in-vitro' dan perkembangan anak pokok daripada embrio zigotik kelapa sawit (variati Dura dan hibrid Tenera). Embrio zigotik telah dikultur dengan menggunakan medium Murashige dan Skoog (MS) yang telah ditambah dengan campuran 0.05 atau 0.1 mgL<sup>-1</sup> bagi setiap PGR (asid gibberellik, 6-benzilaminopurine dan asid  $\alpha$ -naphthaleneacetic) dengan atau tanpa 2 gL<sup>-1</sup> AC. Pertumbuhan dan

perkembangan embrio telah didapati dipengaruhi oleh komposisi medium yang digunakan. Embrio zigotik yang telah dikultur dengan menggunakan media MS yang telah ditambah dengan PGR dan AC membantu pertumbuhan pucuk dan perkembangan anak pokok selanjutnya, manakala medium MS yang ditambah PGR tanpa AC membawa kepada pertumbuhan yang tidak normal. Ini membuktikan bahawa AC sangat diperlukan bagi pertumbuhan anak pokok kelapa sawit '*in-vitro*'. Medium terbaik bagi pertumbuhan dan perkembangan anak pokok adalah medium MS yang telah ditambah dengan  $0.1 \text{ mgL}^{-1}$  PGR dan  $2 \text{ gL}^{-1}$  AC memberi perbezaan yang ketara berbanding formulasi medium lain. Medium tersebut telah digunakan dalam kajian seterusnya mengenai kesan pengeringan terhadap kemandirian embrio zigotik yang telah dikrioawet. Embrio zigotik variasi Dura dan hibrid Tenera dikeringkan selama 8 jam. Selepas pengeringan dan pembekuan embrio zigotik kandungan air bebas dan kebolehnya untuk hidup telah dianalisis. Peratus kemandirian maksimum (Dura 80%) dan (Tenera 70%) telah dicatatkan. Embrio zigotik yang dikeringkan sehingga mengandungi  $\sim 0.14 \text{ gH}_2\text{O g}^{-1}$  fw air bebas menyebabkan pertumbuhan yang tidak normal atau mencatatkan peratus kemandirian sifar. Air terbebas yang optimum ini bukan sahaja membantu embrio zigotik untuk mengekalkan integriti sel tetapi juga mengekalkan kebolehan untuk tumbuh selepas dikrioawet. Embrio zigotik yang mengandungi air bebas melebihi  $0.24 \text{ gH}_2\text{O g}^{-1}$  dw atau kurang daripada  $0.16 \text{ gH}_2\text{O g}^{-1}$  dw menyebabkan kehilangan kebolehidupan serta integriti sel akibat air bebas yang



berlebihan atau kehilangan air bebas yang terlalu banyak, selepas proses pengeringan dan pembekuan yang berturutkali. Kajian mikroskopi elektron pengimbas mengesahkan bahawa tiada perbezaan yang ketara bagi morfologi lapisan epidermis selepas pengeringan dan pembekuan yang berturutan. Oleh itu, untuk mengkrioawet kelapa sawit, embrio zigotik perlu dikeringkan sehingga mencapai air bebas sebanyak  $0.14 \text{ gH}_2\text{O g}^{-1} \text{ fw}$  dengan catatan peratus kemandirian sebanyak 80%. Kajian berkenaan kesan pelbagai jenis larutan muatan (LS) dan larutan pemvitreusan (VS) dan juga tempoh masa rendaman terhadap kemandirian poliembrioids dalam nitrogen cecair (LN) telah dilakukan. Poliembrioid yang dikultur '*in-vitro*' telah berjaya dikrioawet secara pemvitreusan dengan peratus kemandirian sebanyak 45%. Poliembrioid individu yang telah diasingkan dan dipisahkan daripada kultur berumpun yang berumur dua bulan (daripada poliembrioid permulaan), telah diprakultur dalam medium MS cecair yang telah ditambah dengan 0.5 M sukrosa selama 12 jam pada suhu  $26 \pm 2^\circ\text{C}$ . Kemudian ia diperlakukan dengan campuran 10% (w/v) DMSO yang telah ditambah 0.7 M sukrosa LS selama 30 minit pada suhu  $26 \pm 2^\circ\text{C}$ . Poliembrioid yang dilindungi secara osmotik pada mulanya diperlakukan dengan menggunakan VS (PVS2 30% (w/v) gliserol ditambah dengan 15% (w/v) EG, 15% (w/v) DMSO dan 0.4 M sukrosa) selama 5 minit pada suhu  $26 \pm 2^\circ\text{C}$ . Selepas penukaran larutan PVS2 yang baru, polyembrioid dimasukkan terus ke dalam LN. Selepas pemanasan pantas dalam rendaman air suam bersuhu  $38 \pm 2^\circ\text{C}$  selama 90 saat, poliembrioid dicuci

selama 20 minit pada suhu  $26 \pm 2^{\circ}\text{C}$  dengan medium MS cair yang mengandungi sukrosa 1.2 M. Ia dipindahkan ke medium MS pepejal yang mengandungi 3% (w/v) sukrosa dan agar 0.75% (w/v). Poliembrioid disimpan dalam keadaan gelap selama tujuh hari sebelum didedahkan kepada cahaya (16 jam kitaran cahaya). Pertumbuhan pucuk diperhatikan pada kira-kira tiga minggu selepas kultur. Secara keseluruhan, medium yang terbaik untuk pertumbuhan embrio zigotik ialah MS dengan  $0.1 \text{ mg}^{-1}$  PGR dan  $2 \text{ gL}^{-1}$  AC dan kira-kira  $0.14 \text{ gH}_2\text{O g}^{-1}$  fw air bebas menunjukkan kemandirian yang tertinggi selepas dikrioawet bagi kedua-dua Dura dan Tenera; 12 h sukrosa prakultur Poliembrioid dirawat dengan L5, 30 minit dan PVS2, 5 minit telah berjaya dikrioawet dalam kajian ini.

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I certify that a Thesis Examination Committee has met on .....2011 to conduct the final examination of Suranthran Periasamy on his thesis entitled "Cryopreservation of Oil Palm (*Elaeis guineensis* Jacq.) for Germplasm Conservation" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee are as follows:

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## DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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**SURANTHRAN PERIASAMY**

Date: 12 April 2012

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