EMBRYOGENIC CALLUS FORMATION IN LOCAL COCOA (*Theobroma cacao* L.) CLONES

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment 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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Chairman: **Associate Professor Radzali Muse, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

A study on the potential of embryogenic callus formation in KKM 1,KKM 15, KKM 17, KKM 22, KKM 27, KKM 28, PBC 123, PBC 159, MHP 78,MHP 79, MHP 296, AMAZ, GS 29, EET 339 and MJS 47 local cocoa clones which had been released by MARDI was carried out. A sigmoid curve for callus growth, viability and total protein content of stem, leaf, matured and immatured cotyledon derived callus were obtained. Based on this curve, the subculture interval was determined and it shows that the appropriate timing or period for subculture was between 20 to 24 days after the incubation. Obviously in this interval, the growth of callus was enormous and the total soluble protein was the highest. However, the viability was slowly decreased due to browning of the callus. Therefore only the white and friable callus was selected for subcultures.

Embryogenic callus was successfully induced for PBC 123 and MHP 296 cocoa clones by using immature cotyledon derived callus. Negative results were obtained

for all clones and explants tested using MS as the embryogenic induction medium but positive results were obtained for PBC 123 and MHP 296 clones using DKW and WPM basic media. The combinations of 2 mg/L 2,4-D and 0.25 mg/L Kin was the most successful auxin and cytokinin combination for embryogenic callus induction.. The nodular embryogenic callus of PBC 123 and MHP 296 emerged after the second subculture. Browning of callus occurred during incubation especially at the end of the culture period and was obvious for both clones. Enhancement of the embryogenic induction media by adding nitrogen rich compounds and amino acid such as casein hydrolysate, malt extract and proline did not improve the initiation of embryogenic callus. Instead, more friable callus were obtained from these enhanced media.

In direct somatic embryogenesis, 15 cocoa clones had been tested and four clones showed positive results. These clones (MJS 47, GS 29, PBC 123 and PBC 159) were able to produce embryogenic callus from staminodes explants using the protocol previously developed by Li et al (1998). The nodular embryogenic callus of MJS 47, PBC 123, PBC 159 and GS 29 emerged at the cut ends and were transferred onto a secondary callus growth medium and continued to proliferate on this medium.

Maturation of the embryogenic callus was carried out in a medium described by Li et al. (1998). The globular callus were pale brown in colour at the early maturation period but turned dark brown after 3 weeks of incubation period. None of the globular callus turned to heart shaped somatic embryo which is the second stage of somatic embryogenesis. The maturation of the globular callus was not achieved and the

embryogenic callus became brown and turned non viable at the end of the incubation period. All embryogenic clones showed similar response to the maturation medium.

Total soluble polyphenol content of embryogenic callus of PBC 123 after 16 days of incubation was 259.94 ± 15.53 μg/g fresh weight which was relatively lower than the non embryogenic callus (451.19 ± 5.42 μg/g fresh weight). The increment or accumulation of the TSP was almost similar in MHP 296 immatured cotyledon derived callus especially towards the end of the incubation period. Furthermore, the embryogenic callus which were induced from immature cotyledon of MHP 296 and PBC 123 showed high polyphenol accumulation as compared to embryogenic callus induced from staminode of GS 29, PBC 123, PBC 159 and MJS 47. The browning of non embryogenic callus was greater than embryogenic callus. The specific peroxidase activity was slowly increased as the callus became embryogenic. The POD activity was 164.27 ± 9.42 unit/mg protein for GS 29 embryogenic callus while for non embryogenic callus, the activity was around 63.31 ± 9.24 unit/mg protein at 16 days of incubation. Even though a similar pattern of increment could be observed between the embryogenic and non embryogenic callus, the POD activities was higher in embryogenic callus as compared to non embryogenic callus.

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

PENGARUHAN KALUS EMBRIOGENIK BAGI KLON KOKO (*Theobroma cacao* L.)TEMPATAN

Oleh

ASFALIZA RAMLI

Mac 2006

Pengerusi: **Profesor Madya Radzali Muse, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Kajian mengenai potensi klon koko tempatan yang telah diisytiharkan oleh MARDI untuk menghasilkan embrio somatic telah dilakukan. Keluk sigmoid bagi pertumbuhan, kebolehidupan dan kandungan protein kalus dari batang, daun, kotiledon tidak matang dan kotiledon diperolehi. Berdasarkan keluk ini, sela tempoh untuk pengkulturan semula ialah diantara 20 hingga 24 hari selepas inkubasi. Sela tempoh ini jelas menunjukkan pertumbuhan kalus adalah tertinggi dan kandungan protein adalah tertinggi. Walau bagaimanapun, kebolehidupan kalus semakin berkurang disebabkan proses pencoklatan. Dengan itu, hanya kalus yang peroi dan berwarna putih sahaja dipilih untuk pengkulturan semula.

Pengaruhan kalus embriogenik telah berjaya dilakukan untuk klon koko PBC 123 dan MHP 296 dengan menggunakan kalus yang terbentuk dari kotiledon tidak matang. Keputusan negatif bagi pengaruhan kalus embriogenik ditunjukkan oleh semua klon koko dan eksplan yang dirawat dengan media MS. Keputusan yang positif didapati bagi

klon koko PBC 123 dan MHP 296 dengan menggunakan media DKW dan WPM sebagai media asas. Kombinasi 2 mg/L (w/v) 2,4-D dan 0.25 mg/L(w/v) Kin merupakan kombinasi auksin dan sitokinin terbaik bagi pengaruhan kalus embriogenik. Kalus embryogenik nodular bagi klon PBC 123 dan MHP 296 mula kelihatan semasa pengkulturan semula yang ke dua. Kalus menjadi keperangan semasa dalam tempoh pengeraman terutama sekali pada akhir tempoh pengeraman bagi kedua dua klon tersebut. Penambahbaikan komposisi media dengan penambahan beberapa jenis kompaun kaya nitrogen dan asid amino seperti kasein hidrolisat, ekstrak malta dan prolin didapati tidak membantu di dalam pengaruhan kalus embriogenik. Walaubagaimana pun penambahan kompaun ini dapat menjadikan kalus lebih peroi.

Bagi pengaruhan embryo secara langsung, 15 jenis klon telah diuji tetapi hanya 4 jenis sahaja yang menunjukkan hasil positif. Klon-klon MJS 47, GS 29, PBC 123 dan PBC 159 berupaya menghasilkan kalus embriogenik daripada eksplan staminod dengan menggunakan protokol yang telah dibangunkan oleh Li et al., (1998). Kalus embriogenik nodular bagi klon – klon MJS 47, GS 29, PBC 123 dan PBC 159 kemudiannya dipindahkan ke media pertumbuhan sekunder untuk perkembangan kalus selanjutnya.

Pematangan kalus embriogenik dilakukan dengan menggunakan media yang dibangunkan oleh Li et al., (1998). Kalus embriogenik nodular berwarna coklat pucat semasa awal pematangan dan bertukar ke coklat gelap selepas tiga minggu tempoh pengeraman. Kesemua kalus embriogenik gagal melepasi tahap pembentukan embrio soma berbentuk hati yang merupakan tahap ke dua proses pengaruhan embrio somatik.

Proses pematangan kalus embriogenik gagal dimana ianya menjadi keperangan dan hilang kebolehidupan pada akhir tempoh pengeraman. Kesemua klon menunjukkan tindakbalas yang sama terhadap media pematangan.

Kandungan keseluruhan fenol bagi kalus embriogenik bagi klon PBC 123 selepas 16 hari pengeraman adalah 259.94 ± 15.53 μg/g berat basah dimana ianya lebih rendah berbanding kalus tidak embriogenik (451.19 ± 5.42 μg/g berat basah). Kenaikan atau pengumpulan kandungan polifenol ini didapati lebih kurang sama dengan klon MHP 296 yang menggunakan kalus dari kotiledon tidak matang sebagai eksplan terutamanya pada akhir tempoh pengeraman. Disamping itu, kalus embriogenik yang teraruh daripada eksplan kotiledon tidak matang menunjukkan kandungan polifenol yang tinggi berbanding kalus embriogenik dari eksplan staminod. Proses pencoklatan kalus yang tidak embriogenik adalah lebih teruk berbanding kalus embriogenik. Aktiviti enzim peroksida di dapati bertambah secara perlahan-lahan apabila kalus menjadi embriogenik. Aktiviti enzim peroksida bagi kalus embriogenik klon GS 29 ialah 164.27 ± 9.42 unit/mg protein berbanding 63.31 ± 9.24 unit/mg protein bagi kalus tidak embriogenik pada 16 hari selepas pengeraman. Walaupun corak peningkatan aktiviti enzim peroksida yang seakan sama bagi kalus embriogenik dan kalus yang tidak embriogenik, didapati aktiviti enzim lebih tinggi bagi kalus embriogenik berbanding kalus tidak embriogenik.

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I certify that an Examination Committee has met on 3rd March 2006 to conduct the final examination of Asfaliza Ramli on her Master of Science thesis entitled "Embryogenic Callus Formation in Local Cocoa (*Theobroma cacao* L.) Clones" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as partial fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions

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TABLE OF CONTENTS