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SUSPENSI SEL PADA KELAPA SAWIT (*Elaeis guineensis*, Jacq)

Gale Ginting dan Fatmawati

ABSTRAK

Produksi klon kelapa sawit secara kultur jaringan saat ini masih menghadapi beberapa kendala yaitu rendahnya indeks perbanyakan kultur pada tahap kalus, embrio, dan pupus. Semua tahap pada proses kultur jaringan menggunakan media padat sehingga biaya produksinya relatif mahal. Suspensi sel merupakan pengembangan teknik kultur jaringan yang menggunakan medium cair untuk menghasilkan plantlet. Pada suspensi sel dilakukan induksi embrio somatik dari friable callus. *Selanjutnya dari embrio somatik dihasilkan pupus dalam medium cair. Penelitian suspensi sel menggunakan modifikasi medium dasar MS (Murashige T & Skoog F, 1962) dengan penambahan karbon aktif 2 g/l medium dan perlakuan hormon* 2, 4-dichlorophenoxyacetic acid *pada lima dosis, masing-masing: 50, 25, 10, 5 dan 1 mg/l 2,4-D. Penelitian dilakukan pada 16 jenis klon dari 10 persilangan. Hasil penelitian menunjukkan bahwa hanya pada medium yang mengandung 50 mg /l 2,4-D berhasil didapatkan embrio somatik dan pupus. Sedangkan perlakuan pada jenis medium lainnya gagal mendapatkan embrio somatik maupun pupus. Kualitas pupus yang diperoleh dari medium yang mengandung 50 mg /l 2,4-D cukup baik dan dapat menghasilkan planlet.*

Kata kunci: ***Elaeis guineensis*** Jacq, suspensi sel, *friable callus*, embrio somatik, planlet

PENDAHULUAN

Kelapa sawit termasuk kelompok tanaman monokotil yang sulit diperbanyak secara vegetatif. Produksi klon kelapa sawit secara kultur jaringan telah berhasil dilakukan tetapi prosesnya panjang dan lama karena harus melalui tahapan-tahapan: induksi kalus, embriogenesis, pematangan embrio, induksi pupus, dan perakaran. Di samping itu perbanyakan klon kelapa sawit secara kultur jaringan masih menghadapi beberapa kendala teknis antara lain: lamanya waktu yang dibutuhkan sejak tahap awal eksplan sampai menghasilkan planlet (16-24 bulan), rendahnya indeks perbanyakan pada tahap kalus, embrio, maupun pupus, dan tingginya biaya produksi karena pada semua tahap menggunakan media padat. Biaya produksi planlet yang dihasilkan secara kultur jaringan sekitar US$ 5 per planlet (1, 5).

Salah satu cara untuk mengatasi masalah dalam perbanyakan vegetatif melalui kultur jaringan adalah dengan kultur suspensi. Prinsip teknologi suspensi sel cukup sederhana yaitu dengan menggunakan *friable callus* dari kalus primer untuk membentuk embrio somatik yang bersifat bipolar (ada plumula dan radikula) dalam medium cair. Teknologi ini secara teknis lebih praktis dibandingkan kultur jaringan biasa, karena tahapan proses pembentukan planlet tidak melalui semua proses kultur jaringan seperti tersebut di atas. *Friable callus* langsung menghasilkan embrio somatik yang bersifat bipolar, yang langsung

dapat menghasilkan pupus. Biaya produksi lebih murah karena pada suspensi sel selalu digunakan medium cair, kecuali pada tahap perakaran. Biaya produksi planlet kelapa sawit dengan menggunakan teknologi suspesi sel hanya US$ 0,20/planlet (6) dibandingkan kultur jaringan sekitar US$ 5/planlet. Waktu yang dibutuhkan sejak tahap awal *friable callus* sampai menghasilkan planlet relatif lebih singkat yakni sekitar 8-10 bulan. Kunci sukses produksi planlet melalui suspensi sel adalah tersedianya *friable callus* dalam jumlah yang mencukupi, kemampuan *friable callus* menghasilkan embrio somatik, dan kemampuan setiap sel embrio somatik tumbuh menjadi pupus. Beberapa masalah yang ada pada suspensi sel kelapa sawit antara lain sulitnya membuat stok embrio dan tingginya persentase tanaman abnormal di lapangan; seperti di LaMe yang mencapai 85-90% (6). Penggunaan hormon *2,4-D (dichlorophenoxyacetic acid)* yang tinggi (> 100 mg/l) di dalam medium diduga menjadi penyebab abnormalitas (4). Penelitian ini bertujuan untuk menguji dosis 2,4-D pada level yang lebih rendah (50; 25; 10; 5 dan 1 mg/1 medium).

BAHAN DAN METODE

Untuk menghasilkan embrio somatik dari *friable callus* digunakan lima tingkat dosis 2,4-D yaitu: 50; 25; 10; 5 dan 1 mg/l dengan memakai modifikasi medium dasar Murashige & Skoog (1962) ditambah arang aktif 2 g /l. Sampel yang digunakan adalah *friable callus* 16 jenis klon dari 10 persilangan: BJ126D x LM002T, BJ033D x LM005T, TI006D x RS012T, LM2742D x LM2399P, DS029D x LM451T, BJ019D x LM312P, BJ007D x LM002T, DS139D x NI002P, LM270D x LM239T dan MA284D x LM312P. Jumlah sampel *friable callus* sama untuk setiap perlakuan. Sebagai ulangan percobaan adalah klon. *Friable callus* dimasukkan ke dalam erlenmeyer volume 250 ml berisi medium cair yang telah mengandung perlakuan 2,4-D. Kultur digoyang di atas *shaker* dengan kecepatan 85-90 rpm di ruang kultur dengan intensitas cahaya 1000 *gross lux* selama 12 jam/hari. Pengamatan kultur dilakukan satu kali seminggu dan subkultur satu kali setiap dua minggu. Setelah 2-3 bulan, *friable callus* akan menghasilkan embrio somatik. Selanjutnya, embrio somatik dipanen dari medium cair dan ditanam dalam media padat tanpa 2,4-D. Penanaman ini bertujuan untuk proses pematangan embrio somatik yang membutuhkan waktu 1-2 bulan. Embrio somatik kemudian dikultur dalam medium baru untuk penumbuhan daun dan akar. Peubah yang diamati adalah jumlah embrio somatik yang terbentuk dari *friable callus* di dalam erlenmeyer dan jumlah pupus/planlet yang dihasilkan dari embrio somatik di dalam tes tubes sesuai perlakuan.

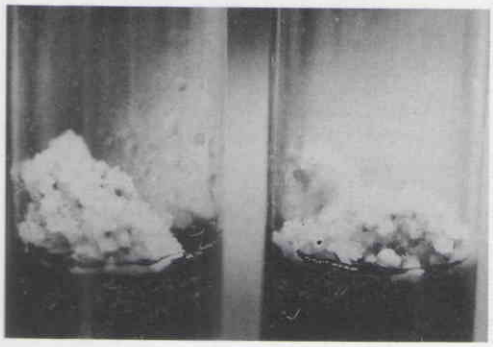
HASIL DAN PEMBAHASAN

Induksi embrio somatik dari *friable callus* dengan dosis perlakuan 1 - 25 mg /1 2,4-D tidak berhasil untuk semua klon. *Friable callus* mengalami pencoklatan setelah 1 - 2 bulan dikultur. Pada sub-kultur siklus ke dua *friable callus* mengalami nekrosis dan mati. Kalus memerlukan hormon 2,4-D untak pembelahan sel dan perkembangannya (2,3), sedang-

Tabel 1. Respon *friable callus* terhadap perlakuan 2,4-D

Klon (MK)	F.C. (E)	2,4-D (mg/l)									
		50		25		10		5		1	
		E.S (E)	Pupus (T)	E.S (E)	Pupus (T)	E.S (E)	Pupus (T)	E.S (E)	Pupus (T)	E.S (E)	Pupus (T)
122	3	4	1	0	0	0	0	0	0	0	0
123	18	8	8	0	0	0	0	0	0	0	0
138	12	7	1	0	0	0	0	0	0	0	0
145	4	2	1	0	0	0	0	0	0	0	0
178	14	8	7	0	0	0	0	0	0	0	0
185	8	5	2	0	0	0	0	0	0	0	0
186	2	4	1	0	0	0	0	0	0	0	0
188	3	1	0	0	0	0	0	0	0	0	0
192	6	6	10	0	0	0	0	0	0	0	0
199	8	6	5	0	0	0	0	0	0	0	0
203	2	1	0	0	0	0	0	0	0	0	0
221	7	4	1	0	0	0	0	0	0	0	0
225	9	4	3	0	0	0	0	0	0	0	0
228	7	4	0	0	0	0	0	0	0	0	0
232	4	2	1	0	0	0	0	0	0	0	0
237	11	6	2	0	0	0	0	0	0	0	0
Total	118	72	43	0	0	0	0	0	0	0	0

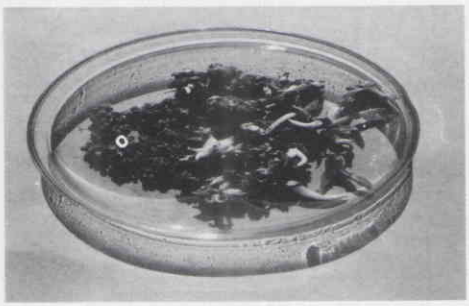
Keterangan : MK = Marihat Klon, FC = *friable callus*. ES = embrio somatik. E= erlenmeyer, T = *test tube*



Gambar 1. *Friable callus*

Gambar 2. Induksi embrio somatik dari *friable callus*

kan karbon aktif menyerap hormon 2,4-D. Menurut Verdeil *et al.*(7), hanya 0,47% hormon 2,4D yang tertinggal di dalam medium yang mengandung arang aktif 2-3 g /l. Dosis 2,4-D yang sangat rendah di dalam medium diduga diserap oleh arang aktif, sehingga jumlahnya tidak mencukupi untuk kebutuhan pembelahan sel maupun perkembangan kalus. Akibatnya, terjadi kalus mengalami pencoklatan, nekrosis, dan mati. Hasil penelitian ini menunjukkan bahwa suspensi sel yang terbaik diperoleh pada perlakuan 50 mg /l 2.4-D.



Gambar 3. Embrio somatik dan pupus

Pada perlakuan ini semua sampel dari 16 jenis klon dapat menghasilkan embrio somatik dari *friable callus*. Pembentukan embrio somatik memerlukan waktu 2-4 bulan. Embrio somatik yang dihasilkan berjumlah 72 erlenmeyer (Tabel 1). Pematangan embrio somatik membutuhkan waktu 2-3 bulan. Embrio somatik yang matang berbentuk oval bersifat bipolar. Sejumlah 43 tabung pupus telah dihasilkan oleh seluruh klon, kecuali klon MK 188. 203 dan 228. Hasil suspensi sel dengan perlakuan: 50; 25; 10; 5 dan 1 mg/l 2,4-D disajikan pada Tabel 1.

Pupus yang dihasilkan pada perlakuan 50 mg/l 2,4-D cukup jagur dan secara morfologis bentuk dan warnanya sangat baik. Namun demikian, kualitas klon hasil suspensi sel ini perlu diuji lebih lanjut di lapang.

KESIMPULAN DAN SARAN

Medium yang mengandung 50 mg/ l 2,4-D dapat menghasilkan embrio somatik pupus/planlet. Untuk mengetahui kualitas klon hasil suspensi sel, perlu dilanjutkan uji lapang. Pada penelitian ini biaya produksi planlet belum dapat dihitung secara rinci namun diduga dapat bersaing dengan biaya produksi benih secara konvensional. Apabila klon yang dihasilkan melalui suspensi sel kualitasnya telah teruji baik di lapangan, maka pengembangan suspensi sel merupakan solusi untuk menghasilkan klon kelapa sawit secara komersial.

DAFTAR PUSTAKA

1. ALAIN, R., Y. DUVAL, J.L. VERDEIL and F. ABERLENC. 1993. Recent advances in oil palm *(Elaeis guineensis,* Jacq.) clonal propagation. Application of Plant *in-vitro* Technology. pp: 156-163

2. BLANDINE de TOUCHET, Y. DUVAL and C. PANNETIER. 1991. Plant regeneration from embryogenic suspension cultures of oil palm *(Elaeis guineensis,* Jacq). Plant cell Reports 10: 529-532

3. BLANDINE de TOUCHET, A. NATOO, L. DANIELE and Y. DUVAL. 1993. Liquid medium and carboxylase activities in oil palm propagated *in-vitro.* Plant Physiol. Biochem. 31(6): 1-5

4. CORLEY, R.H.V., C.H. LEE, I.H. LAW and C.Y. WONG. 1986. Abnormal flower development in oil palm clones. Planter, 62:233-240

5. CORLEY, R.H.V. 1991. Fifteen years experience with oil palm clones - A Review of progress. *In* proceedings of the 1991 PORIM International Palm Oil Conference, Kuala Lumpur, pp 69-81

6. DUVAL, Y., F. ABERLENC and BLANDINE de TOUCHET. 1993. Use of embryogenic suspensions for oil palm propagation. In proceedings of the 1993 ISOPB International Symposium on Recent Developments in Oil Palm Tissue Culture And Biotechnology. Kuala Lumpur. pp: 38-47

7. VERDEIL, J.L., C. HUET and Y. DUVAL. 1993. Adsorption of 2,4-D Dichlorophenoxyacetic Acid by activated charcoal in plant tissue culture media. CIRAD-CP, France.

Cell suspension on oil palm (*Elaeis guineensis*, Jacq)

Gale Ginting and Fatmawati

Abstract

Oil palm micropropagation is hampered by some limitations, such as low multiplication indeces in callus, embryo, and shoot stages. The use of solid media in these stages makes high production cost. In an attempt to overcome the limitations, a technique using liquid medium (cell suspension) was developed. In this technique, somatic embryo induced from friable callus was used to produce shoots. An experiment was undertaken to select the optimum concentrations of 2,4-D (1 to 50 mg/l) in a modified MS (Murashige T. & Skoog F., 1962) liquid medium containing activated charcoal (2 g/l) using 16 clones of 10 crosses. The results showed that somatic embryos and shoots could only be produced by the medium containing 50 mg/l 2,4-D. The shoots having good quality and could produce plantlets. The rest of the concentranons tested failed to produce somatic embryos.

Key words : *Elaeis guineensis* Jacq, cell suspension, friable callus, somatic embryo, plantlet

Introduction

Oil palm is a monocot which is recalcitrant to vegetative propagation. Clone production through tissue culture has been in operational for about 15 years although the process is long and time consuming as it passes many stages. These include callus induction, embryogenesis, embryo maturation, shoot formation and rooting. The process may take 16 to 24 months. In addition to time constraint, the production is hampered by technical problems including low multiplication indices at callus, embryo and shoot formation stages. These lead to a high cost production particularly due to that all stages use solid media. The cost is estimated at about US$ 5 per plantlet (5).

An alternative to overcome the problems is the use of cell suspension. The principle is simple that is to use friable callus derived from primary callus to produce bipolar somatic embryos (having plumule and radicle) in a liquid medium. The technique is more practical because the suspension culture can skip some of the stages required in the solid-based tissue culture. The friable callus will directly produce bipolar somatic embryos which then germinate. The production cost decreases since the media devoid agar except for rooting stage. The cost of production is estimated at only US$ 0.20 per plantlet (6) compared to US$ 5 in the ordinary tissue culture system. In addition, the time required to produce plantlet is only 8 to 10 months starting from friable callus. The key factors of the cell suspension are (a) the availability of friable callus, (b) the capacity of friable callus to produce somatic embryos, and (c) the viability of the embryos. Some of the problems currently faced within the cell suspension system are difficulty to produce stock of embryos and high clone abnormality in the field, for example 85-90% in La Me (6). The use of dichlo-

rophenoxyacetic acid (2.4-D) at high level (>100 mg/l) may cause the abnormality (4). The present study, therefore, was to select the optimum concentration of 2,4-D at lower levels.

Materials and Methods

Five dosages (50, 25, 10, 5 or 1 mg/l) of 2,4-D were tested to produce somatic embryos from friable callus using a modified MS medium (Murashige & Skoog, 1962) supplemented with activated charcoal (2 g/l) on sixteen clones of ten crosses. They were BJ126D x LM002T, BJ033D x LM005T, TI006D x RS012T, LM2742D x LM2399P, DS029D x LM451 T. BJ019D x LM312P, BJ007D x LM002T, DS 139D x NI002P, LM270D x LM239T and MA284D x LM312P. The amount of friable callus used was equal in all treatments and replications. The callus was placed in 250 ml erlenmeyer flasks containing liquid medium with 2,4-D of different concentrations as previously described. The cultures were shaken (85-90 rpm) in an illuminated room (1000 gross lux, 12 h per day). Observation and subculture were undertaken every week and fortnightly, respectively. The callus produced somatic embryos 2 to 3 months later and the embryos were harvested, placed on solid medium without 2,4-D in test tubes and cultured for 1 to 2 months to mature the embryos. Further subculture was undertaken for shoot formation and rooting. Observations were made to see the number of somatic embryos produced in the flasks, shoots or plantlets germinated from the embryos in the test tubes.

Results and Discussion

There was no induction of somatic embryos from the callus when the medium contained 1 to 25 mg/l 2,4-D. The callus turned brown in 1 to 2 months after the start of culture. They then necrosed and died in the subsequent subcultures. Callus requires 2,4-D for cell division and its development (2, 3). In the present study the amount 2,4-D given at less than 25 mg/l may not be sufficient since the activated charcoal absorb a high proportion of this plant growth regulator. It has been known that only 0.47% of a given 2,4-D is available in a medium containing 2-3 g/l of activated charcoal (9). Insufficient 2.4-D in the medium may cause the callus necrosed and died. At higher concentration (50 mg 1 I), however, 2,4-D produced somatic embryos in all clones. This process occurred within 2 to 4 months. There were 72 flasks producing the embryos (Table 1). These embryos matured in 2 to 3 months as indicated by bipolarity and oval in shape. They produced 43 tubes of shoots in all clones except MK188, 203 and 228. The shoots produced were good as indicated by their morphological appearances. However, these clones should be planted in the field to assess their quality.

Conclusion and Suggestions

It is necessary to add 50 mg/l 2,4-D into the modified MS liquid medium to produce somatic embryos which then yield shoots or plantlets. The study should be continued to assess the quality of the clones in the field. The cost

Table 1. Responses of friable callus on 2,4-D

Clone (MK)	F.C (E).	2,4-D (mg/l)									
		50		25		10		5		1	
		E.S (E)	Shoots (T)	E.S (E)	Shoots (T)	E.S (E)	Shoots (T)	E.S (E)	Shoots (T)	E.S (E)	Shoots (T)
122	3	4	1	0	0	0	0	0	0	0	0
123	18	8	8	0	0	0	0	0	0	0	0
138	12	7	1	0	0	0	0	0	0	0	0
145	4	2	1	0	0	0	0	0	0	0	0
178	14	8	7	0	0	0	0	0	0	0	0
185	8	5	2	0	0	0	0	0	0	0	0
186	2	4	1	0	0	0	0	0	0	0	0
188	3	1	0	0	0	0	0	0	0	0	0
192	6	6	10	0	0	0	0	0	0	0	0
199	8	6	5	0	0	0	0	0	0	0	0
203	2	1	0	0	0	0	0	0	0	0	0
221	7	4	1	0	0	0	0	0	0	0	0
225	9	4	3	0	0	0	0	0	0	0	0
228	7	4	0	0	0	0	0	0	0	0	0
232	4	2	1	0	0	0	0	0	0	0	0
237	11	6	2	0	0	0	0	0	0	0	0
Total	118	72	43	0	0	0	0	0	0	0	0

Notes : MK = Marihat clone, FC = friable callus, ES = Somatic Embryo, E = erlenmeyer, T = test tube

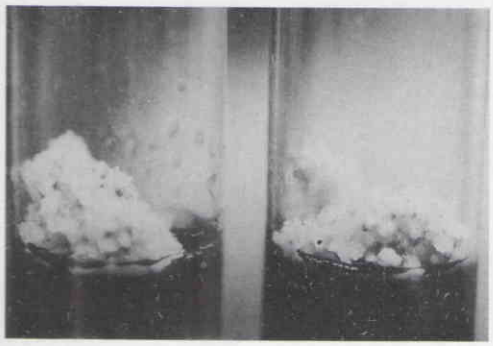


Figure 1. Friable callus

Figure 2. Induction of somatic embryos on friable callus

involved in the production has not been calculated in the present study. However, it could be expected that the cost would match that of seeds produced in seed gardens. Once the fidelity of the palms produced from the suspension system has been tested, clone production on commercial scale could be rationalized.

References

1. ALAIN, R., Y. DUVAL, J.L. VERDEIL and F. ABERLENC. 1993. Recent advances in oil palm (*Elaeis guineensis*, Jacq.) clonal propagation. Application of Plant *in-vitro* Technology. pp: 156-163

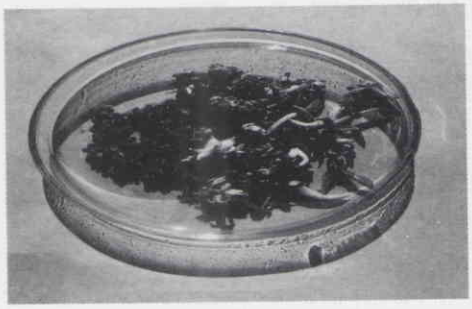


Figure 3. Somatic embryos and shoots

2. BLANDINE de TOUCHET, Y. DUVAL and C. PANNETIER. 1991. Plant regeneration from embryogenic suspension cultures of oil palm *(Elaeis guineensis,* Jacq). Plant cell Reports 10: 529-532

3. BLANDINE de TOUCHET, A. NATOO, L. DANIELE and Y. DUVAL. 1993. Liquid medium and carboxylase activities in oil palm propagated *in-vitro*. Plant Physiol. Biochem. 31(6): 1-5

4. CORLEY, R.H.V., C.H. LEE, I.H. LAW and C.Y. WONG. 1986. Abnormal flower development in oil palm clones. Planter, 62:233-240

5. CORLEY, R.H.V. 1991. Fifteen years experience with oil palm clones - A Review of progress. *In* proceedings of the 1991 PORIM International Palm Oil Conference, Kuala Lumpur, pp 69-81

6. DUVAL, Y., F. ABERLENC and BLANDINE de TOUCHET. 1993. Use of embryogenic suspensions for oil palm propagation. In proceedings of the 1993 ISOPB International Symposium on Recent Developments in Oil Palm Tissue Culture And Biotechnology. Kuala Lumpur. pp: 38-47

7. VERDEIL, J.L., C. HUET and Y. DUVAL. 1993. Adsorption of 2,4-D Dichlorophenoxyacetic Acid by activated charcoal in plant tissue culture media. CIRAD-CP, France.

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