
Primary and secondary somatic embryos as tool for the propagation and artificial seed production of oil palm

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This study describes a protocol for producing artificial oil palm seeds using encapsulated somatic embryos (SEs) and encapsulated secondary somatic embryos (SSEs) and compares the outcome of a number of different methods of cloning oil palm. The study found that the best plant material were SSEs which displayed superior germination performance to SEs, all of which failed to germinate, as well as a better conversion rate into plantlets. For encapsulation, the optimum concentration of sodium alginate complexing agent was 2.5%, artificial seeds being produced by the plant material being immersed in 100 mM calcium nitrate solution for 15 minutes and then rinsed three times with MS liquid media (without PGRs). The optimum size at which SSEs converted into plantlets was found to be 3 mm and the conversion rate of encapsulated SSEs at the coleoptile stage was found to be the highest at 73% after being cultured on MS medium supplemented with 200 mg/l ascorbic acid 3% sucrose and 0.75% agar for 1 week. The rate of normal plantlet development 1 month after germination was 4.4%. The final germination rate was 46.5% and the rate at which complete plantlets developed after being cultured for 5 months was 9.4%.

Key words: Artificial seed, oil palm (*Elaeis guineensis* Jacq.), secondary somatic embryo (SSE), cell suspension

Introduction

The clonal propagation of oil palm has been studied for many years as a potential way of developing high-yielding clones while circumventing the long generation time required by traditional breeding techniques. Somatic embryogenesis is a process which has been used successfully with both solid and liquid media from the 1970s onwards (Aberlenc-Bertossi, 1999; Duval *et al.*, 1988; de Touchet *et al.*, 1991; Nwankwo and Krikorian, 1983; Paranjothy and Othman, 1982; Rabechault *et al.*, 1976; Rival *et al.*, 1997; Teixeira *et al.*, 1993, 1995) and has a strong commercial basis. Somatic embryogenesis is

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advantageous for mass propagation, genetic improvement programs and the production of synthetic seeds (Hartmann *et al.*, 1997).

Cell suspension cultures have been used as an alternative technique for oil palm propagation. De Touchet *et al.* (1991) reported the culture of meristematic clumps in a liquid medium for the regeneration of somatic embryos. Teixeira *et al.* (1995) were successful in establishing cell suspensions from long-term maintained friable embryogenic tissue. The advantage of cell suspension culture over callus culture is that culture parameters, such as pH, temperature, dissolved oxygen, and the availability of nutrients can be optimized and controlled. Therefore, it is possible to synchronize the development stage of somatic embryos which can accelerate the mass propagation of oil palm to keep pace with the high demand for oil palm seedlings.

Secondary somatic embryogenesis is a process whereby new somatic embryos are initiated from originally formed somatic embryos or primary somatic embryos. In many species the efficiency of germination of somatic embryos (SEs) in primary embryogenesis is lower than in secondary embryogenesis. This phenomenon has been described in at least 80 Gymnosperm and Angiosperm species (Raemakers *et al.*, 1995). High-frequency plant regeneration systems through secondary embryogenesis have been reported in several plant species (Chen and Chang, 2004; Choi *et al.*, 1997; Das *et al.*, 1997; das Neves *et al.*, 1999; Giridhar *et al.*, 2004; Merkle *et al.*, 1990; Weissinger II and Parrott, 1993). In oil palm, Te-chato and Hilae (2007) and Chehmalee and Te-chato (2008) reported success in inducing a large number of secondary somatic embryos (SSEs) from single haustorium embryos (HEs) and the efficient germination of those SSEs on PGR-free MS medium. The success of somatic embryo production from embryogenic calluses, cell suspension and SSE holds great potential for these materials to be used in artificial seed production.

Synthetic seed production is an applied technology which capitalizes on the capacity for plant multiplication via somatic embryogenesis (Ipekci and Gozukirmizi, 2003). Artificially encapsulated somatic embryos can be sown under *in vitro* or *ex vitro* conditions, producing uniform clones (Aitken-Christie *et al.*, 1995). Synthetic seeds have many advantages over those produced for propagation by organogenesis, including their ease of handling and potential for long-term storage, a higher scale-up potential and their low cost of production (Ghosh and Sen, 1994). Synthetic seed research has now been extended to numerous crops, including conifers (Fowke *et al.*, 1994), high-value vegetable crops like celery, lettuce (Sanada *et al.*, 1993), pistachio (Onay *et al.*, 1996), and woody plants (Bapat and Rao, 1988; Gray, 1987; Rao and Bapat, 1993).

This study, reports on a source of SEs and a simple protocol used for artificial production of oil palm seeds that can be converted into plantlets.

Materials and methods

Plant material

The friable embryogenic calluses (FEC) used in this study were derived from young leaves of elite clones of the oil palm cultivar, tenera (DxP) from the Thepa Research Station, Faculty of Natural Resources, Prince of Songkla University using the protocol described by Te-chato (1998). The calluses were firstly induced on Murashige and Skoog (MS) medium containing 2.5 mg/l 3, 6-dichloro-2-methoxybenzoic acid (dicamba), 200 mg/l ascorbic acid and 3% sucrose and maintained on solidified embryogenic proliferation media (PM) consisting of MS supplemented with 0.1-1.0 mg/l dicamba. Both media were solidified with 7.5 g/l agar and adjusted to pH 5.7 with KOH prior to autoclaving at 121°C for 15 minutes. The FEC was incubated at 28±2°C under a 14 hour photoperiod and subcultured onto fresh medium every 4 weeks for more than 5 years.

Establishment of cell/embryogenic cell suspension

A quantity of 0.5 g fresh weight of FEC was dispersed in liquid MS medium supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid and 3% sucrose. Culturing was performed in Erlenmeyer flasks (125 ml) containing 25 ml of culture media, sealed with aluminum foil and kept on a gyratory shaker at 110 rpm, 26±4°C, under a 14 hour photoperiod at 15 µmol/m²/sec illumination. The subcultures were carried out by collecting the small cell aggregates of approximately 1.5ml packed cell volume (PCV) in a wide-bore 5 ml pipette and transferring them to a new medium of the same composition every 4 weeks during a period of one year.

Embryogenesis was similarly conducted, with quantities of cells in suspension of approximately 1.5 ml PCV being collected as earlier described and transferred to MS medium supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid, 3% sucrose and 0.2 M sorbitol. All the cell suspension cultures were maintained in the same conditions throughout the proliferation phase. Subculturing of the cells in suspension was carried out every 4 weeks for 6 months. Finally, the somatic embryos which developed from those cultures (Figure 1a) were used for the production of artificial seeds or cultured on MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and 0.75% agar

(germination medium) for 6 months to induce the generation of plantlets and SSE.

Induction of SSEs

After three successive subcultures of the somatic embryo material to the germination medium at 4 week intervals, green mature somatic embryos known as haustorium embryos (HEs) were induced. 12 weeks later, SSEs were observed and an average number of 20-25 SSEs per HE were obtained (Figure 1b). SSEs at this stage were asynchronous and could be divided into five stages of development ranging from the youngest to fully mature embryos ready for germination as follows; Clusters of globular embryos (cl), Globular embryos (g), Torpedo embryos (t), Haustorium embryos (h), Coleoptile producing embryos (co). Those embryos were used as the source material for artificial seed production.

Artificial seed production

Encapsulation of SEs derived from embryogenic cell suspension

SEs of 1, 2 and 3 mm in size were used for artificial seed production (Figure 1a). The SEs were first mixed with MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and sodium alginate variously at concentrations of 2.0, 2.5 or 3.0% (w/v), then dropped into a complexing agent (calcium chloride or calcium nitrate solution at concentrations of 100 or 150 mM) using a sterilized pipette. After that, the somatic embryos were left in the complexing agent for 15 or 30 minutes for full polymerization. Next the artificial seeds were rinsed three times with MS liquid media (without PGRs) and were then plated on MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and 0.75% agar (germination medium) for germination and culture. Finally, the conversion rate into plantlets was determined after 5 months.

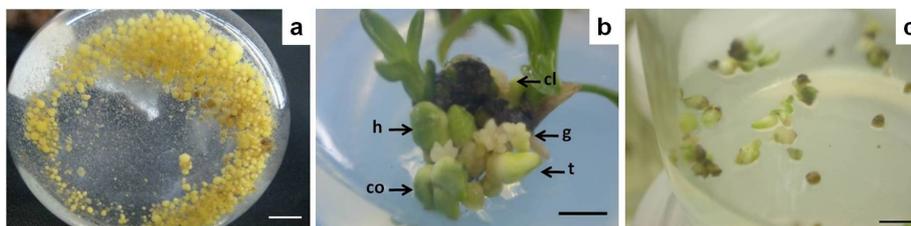


Fig. 1. SEs developed in cell suspension culture (a), SSEs at various stages of development from cultured HEs*in stress medium (b) used for artificial seed production. (bar=0.5cm) and single SSEs from different development stages after being cultured for 1 month (c) (bar=1 cm). g=globular, t=torpedo, co=coleoptile h=haustorium and cl=globular cluster.

Encapsulation of SSEs

SSEs at five developmental stages as mentioned earlier derived from HEs cultured on MS medium supplemented with 200 mg/l ascorbic acid, 0.2 M mannitol and 0.75% agar were separated (Figure 1c) and mixed with MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and 2.5% (w/v) sodium alginate and dropped into 100 mM calcium nitrate solution as a complexing agent, using a sterilized pipette. The SSEs were left in the complexing agent for 15 minutes. Then the artificial seeds were rinsed three times with MS liquid media (without PGRs) and were then plated on germination medium for culture. Finally, the conversion rate of the seeds into plantlets was determined after 5 months.

Results

The effect of different concentrations of sodium alginate and different complexing agents on artificial seed production

Three different concentrations of sodium alginate were used in this study as well as two different complexing agents, and the time of exposure to the complexing agent during artificial seed production was also different. It was found that 2.5% sodium alginate and 100 mM calcium nitrate for 15 minutes was the most effective combination of these three variables, for encapsulating the artificial seeds. A lower concentration (2%) of sodium alginate resulted in beads which were too soft and were difficult to handle whereas with higher a concentration of sodium alginate (3%), the beads were too hard. The concentration and immersion time of the complexing agent also affected the encapsulation of the synthetic seeds. Calcium chloride and calcium nitrate at both concentrations (100 or 150 mM) gave the same performance of bead formation and an exposure time at 15 minutes was found to be the most suitable for encapsulation (Figure 2).

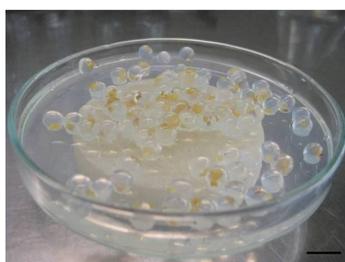


Fig. 2. Encapsulation of somatic embryos of oil palm with MS medium supplemented with 200 mg/l ascorbic acid 3% sucrose and 2.5% (w/v) sodium alginate and soaked in 100 mM calcium nitrate solution for 15 minutes. (bar = 1 cm)

Survival and conversion into plantlets of encapsulated SE artificial seeds

None of the somatic embryos encapsulated with calcium chloride survived. Small sized (1-2 mm) SEs turned brown and died after culture on the germination medium within 1 week. Larger SEs (3 mm) turned brown and died after being cultured for 1 month (Figure 3a).

On the other hand, all the SEs encapsulated with calcium nitrate survived irrespective of their size. SEs of 2 and 3 mm turned green within 3 months of being cultured on germination medium. However, they did not germinate into plantlets (Figure 3b).

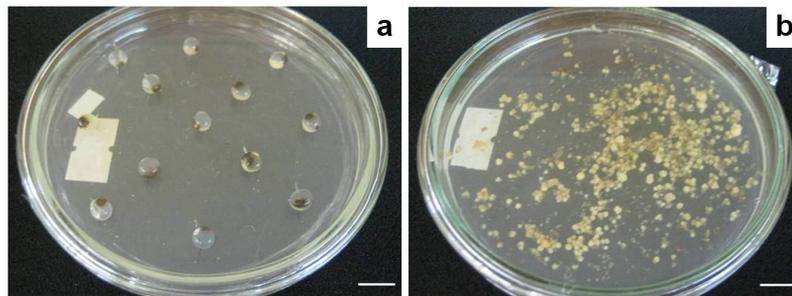


Fig. 3. Encapsulated 3 mm oil palm SEs with 2.5% sodium alginate polymerized with 100 mM calcium chloride for 15 minutes and cultured on MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and 0.75% agar for 1 month (a) and plating of embryogenic cell suspension on MS medium supplemented with 200 mg/l ascorbic acid, 3% sucrose and 0.75% agar for 1 month (b) (bar=1 cm).

Survival and conversion into plantlets of encapsulated SSE artificial seeds

Embryo survival

Many of the encapsulated SSEs treated with 2.5% sodium alginate and polymerized with 100 mM calcium nitrate for 15 minutes (Figure 4a) survived from 1 week to five months of culture although some of them died. Globular cluster embryos survived at the highest rate of 100%, although the survival rate was not significantly different from those obtained from other non-encapsulated embryos. On the other hand, none of the globular embryos survived after being cultured for 1 week (Table 1). The encapsulation of torpedo type SSEs resulted in a severe drop in their survival rate of between 70 and 95%. Although more than 80% of the haustorium embryos survived after being cultured for 1 week there was a severely reduced survival rate of less than 50% 1 month later. However, all types of non-encapsulated SSEs survived at 100%. The present

study suggests that coleoptile embryos are suitable for encapsulation with the highest survival rate of nearly 80% (Table 1).

Table 1. Survival and germination rates of non-encapsulated and encapsulated SSEs after different culturing periods on MS medium supplemented with 200 mg/l ascorbic acid 3% sucrose and 0.75% agar

Type of SSE	Survival rate after different culturing periods (%)			
	1 w	1 m	3 m	5 m
Globular (g)				
Non-encapsulate	100a	100a	0e	0e
Encapsulate	0e	0e	0e	0e
Torpedo (t)				
Non-encapsulate	100a	100a	100a	100a
Encapsulate	31d	17.6d	5.8d	5.8d
Haustorium (h)				
Non-encapsulate	100a	100a	100a	100a
Encapsulate	88c	42.7c	38.8c	38.9c
Coleoptiles (co)				
Non-encapsulate	100a	100a	100a	100a
Encapsulate	95.2b	84.2b	79.9b	79.9b
Globular cluster (cl)				
Non-encapsulate	100a	100a	100a	100a
Encapsulate	100a	100a	100a	100a
F-test	**	**	**	**
C.V.(%)	3.4	5.3	5.1	5.1

** Significant difference at P=0.01

Mean values sharing the same letter in common within a column are not significantly different based on DMRT.

Conversion into plantlets

Only two types of embryos, haustorium and coleoptile were successfully germinated into plantlets. The best results were obtained from coleoptile-stage SSEs (9.4%). They germinated shoots after being cultured on hormone-free MS medium for 1 week (Table 2, Figure 4b). At the end of the first week the coleoptile-stage SSEs had the highest shoot germination rate at 73% out of all the types of encapsulated SSEs. However, some shoots died leading to a gradually decreasing shoot regeneration rate and after being cultured for 5 months the shoot germination rate was 46.5%. The shoot conversion rate for torpedo embryos was less than 10% after being cultured for 1 month, and thereafter, all the shoots died. Only the haustorium embryos gave a constant shoot germination rate of 6.4%. On the other hand, the globular cluster embryos produced a higher shoot germination rate varying from 68% in the

first month of culture to 95.4% after being cultured for 5 months. However, the globular cluster embryos formed only tiny shoots which did not elongate and they did not form normal shoots with roots like those obtained from the coleoptile-stage SSEs (Table 2).

Table 2. Germination characteristic of non-encapsulated and encapsulated SSEs after different culturing periods on MS medium supplemented with 200 mg/l ascorbic acid 3% sucrose and 0.75% agar

Type of SSE	Shoot germination rate after different of culturing periods (%)				Root germination rate after different of culturing periods (%)				Complete germination rate after different of culturing periods (%)			
	1 w	1 m	3m	5m	1 w	1 m	3 m	5 m	1 w	1 m	3 m	5 m
Globular												
Non-encap.	0b	0d	0e	0f	0c	0d	0c	0d	0	0b	0d	0d
Encap.	0b	0d	0e	0f	0c	0d	0c	0d	0	0b	0d	0d
Torpedo												
Non-encap.	0b	0d	0e	55.5c	0c	0d	0c	10.4c	0	0b	0d	10.4c
Encap.	0b	5.8c	0e	0f	0c	5.8c	0c	0d	0	0b	0d	0d
Haustorium												
Non-encap.	0b	0d	55.8	55.8c	0c	0d	13.5	27.2b	0	0b	13.5b	27.2b
Encaps.	1.8b	6.4c	6.4d	6.4e	29.4a	27.2a	31.2a	27.2b	0	0b	0d	6.4c
Coleoptile												
Non-encap.	0b	57.2	68.4	68.4b	0c	0d	36.6	57.2a	0	0b	36.6a	57.2a
Encap.	73.0a	55.8b	51.5c	46.5d	4.7b	13.8b	19.5	23.3b	0	4.4a	5.6c	9.4c
Cluster												
Non-encap.	0b	0d	0e	57.2c	0c	0d	0c	0d	0	0b	0d	0d
Encap.	0b	68.1	95.4	95.4a	0c	4.5c	4.5c	4.5cd	0	0b	0d	0d
F-test	**	**	**	**	**	**	**	**	**	**	**	**
C.V.(%)	19.7	11.2	15.3	11.6	63.3	62.9	45.3	33.2		40.8	44.9	33.0

**Significant difference at P=0.01

Mean values sharing the same letter in common within a column* are not significantly different based on DMRT.

However, contrary results were obtained in regard to root formation from the encapsulated SSEs, and an increase in the root germination rate was observed after longer periods of culture. Both encapsulated haustorium- and coleoptile-stage SSEs produced roots within the first week of culture (Figure 4c). Although a high rate of root production resulted from the two types of SSEs most did not produce shoots, giving rise to a low percentage of complete plantlets (Table 2, Figure 4d). The complete plantlet germination rate from the non-encapsulated SSEs was far better than for the encapsulated SSEs. The coleoptile embryos gave the best result at 57.2% which was significantly higher than the rate produced by the haustorium embryos (27.2%) and the torpedo

embryos (10.4%). None of the other types of embryos were able to be converted into complete plantlets (Table 2).

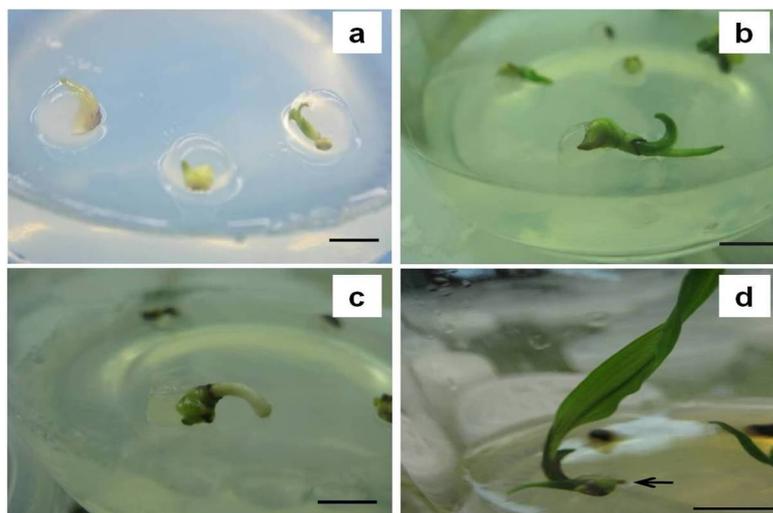


Fig. 4. Encapsulated SSEs of oil palm with 2.5% sodium alginate polymerized in 100 mM calcium nitrate for 15 minutes and cultured on MS medium supplemented with 200 mg/l ascorbic acid 3% sucrose and 0.75% agar. (bar=1 cm) a: Encapsulated SSEs b: Germination of shoot from encapsulated haustorium SSE after 1 month of culture c: Germination of root from encapsulated coleoptile SSEs after 1 week of culture d: Complete plantlet from encapsulated coleoptile SSE after 3 months of culture (arrow showed root)

Discussion

In this study, SSEs were found to be more suitable for use as plant material for artificial seed production than SEs. SEs obtained from cell suspension cultured in MS medium supplemented with 1 mg/l dicamba, 200 mg/l ascorbic acid, 3% sucrose in combination with 0.2 M sorbitol failed to germinate into plantlets after culture on germination medium for 6 months. On the other hand, it was possible to convert SEs into green haustorium somatic embryos and to produce SSEs from them. Only 3 mm encapsulated SEs treated with 2.5% sodium alginate and polymerized in calcium nitrate solution for 15 minutes which were culture on germination medium could be converted into haustorium somatic embryos but the conversion was slower than that of non-encapsulated SEs. This indicates that SEs are not suitable for encapsulation because they are sensitive to sodium alginate and the complexing agents used (calcium chloride and calcium nitrate). Smaller sized somatic embryos were more sensitive than bigger ones.

Moreover, the protocol using calcium chloride as a polymerizing agent affected the survival rate of the somatic embryos. According to Redenbaugh *et al.* (1987), the concentration of the polymerizing agent affects the conversion rate of encapsulated somatic embryos. Ghosh and Sen (1994) achieved a maximum conversion rate of 34% for *Asparagus cooperi* after the encapsulation of individual somatic embryos in 3.5% sodium alginate and polymerization in 50 mM CaCl₂ for 40 minutes. When 75 mM or 100 mM calcium was used, polymerization took between approximately 15 and 20 minutes, but the germination frequency of the encapsulated embryos was significantly reduced. Although in the present study, the polymerizing time was shorter (15 minutes) and the concentrations varied between 100 mM and 150 mM CaCl₂ none of the embryos survived.

The different results in this study may be due to synergistic effects of alginate and the types of calcium compounds used, as well as their concentration. Both alginate and calcium concentrations play a role in the time in which polymerization occurs and capsule hardness is obtained (Redenbaugh *et al.*, 1991). However, a previous study conducted by the authors (unpublished data) detected a reaction between sodium alginate and calcium chloride which produced sodium chloride at high concentrations to form Na⁺ and Cl⁻ ions which severely affects the encapsulated SEs or SSEs.

The SSEs produced better results than the SEs. Te-chato and Hilae (2007) reported that for the micro-propagation of oil palm through callus culture, SSEs induced on 0.2 M sorbitol and 0.1 mg/l dicamba containing full strength MS medium, germinated like normal seedlings (both shoot and root) or shoot alone at a high level of 78%, whereas SEs germinated at only half the level of SSEs (40%). Similarly, the SEs derived from cell suspension cultures used in the present study were not able to germinate into plantlets but were able to produce SSEs which were able to germinate into normal seedlings (57%).

However, the technique of encapsulating the SSEs produced a faster response in coleoptile stage SSEs, followed by haustorium stage SSEs. In this study, encapsulated SSEs at the coleoptile stage were able to germinate a shoot alone at a rate of 73% after being cultured for only 1 week. This phenomenon might be due to the embryos having developed a coleoptile and passing through the haustorium stage and therefore being ready to germinate shoots in a short period. In addition, the encapsulated bead constitutes a source of reserve food similar to an endosperm, necessary for rapid growth and the development of a shoot. Redenbaugh *et al.* (1987) similarly reported that the capsule gel potentially serves as a reservoir for nutrients that may aid the survival and speed the growth of the embryo. The embryos which did not have a well developed shoot forming organ (globular or globular cluster embryos or

torpedo embryos), may need time to develop that organ. The time taken for that organ to develop, in the present study, was at least 1 month. Although the bead consisted of nutrients from the MS medium it also contained a high sodium and calcium salt content which probably had an inhibitory effect on the germination of immature SSEs. Moreover, the SSEs were individually separated by a mechanical method which caused damage to the root meristem and also injured the SSEs. All the SSEs at the globular stage died and those at the torpedo stage germinated shoots at a low level (5.8%).

Generally, shoots grow upward in the direction of the light source as opposed to roots which are geotropic and grow in the direction of gravity. If a developing plant cannot produce a root at the same time as a shoot, it might not be able to take up nutrients and water for further growth and development. For this reason the shoot germination rate of the coleoptile stage SSEs decreased over the culturing period (Table 2) resulting in a low rate of conversion to complete plantlets of 9.4%, whereas the encapsulated SSEs formed roots at an increasing rate over the culture period. This suggests that in SSEs normal geotropic root growth occurs and that SSEs can therefore take up water and nutrients essential for further growth and development. However, in this study although roots were produced widely by healthy SSEs, shoot formation did not always co-occur and only coleoptile and haustorium stage SSEs which formed both a shoot and a root, developed into normal seedlings in germination medium after being cultured for 5 months.

Future experiments should focus on improving the germination rate and the rate of development of complete plantlets based on the composition of the culture medium and the addition of plant growth regulator (PGR) in beads or in the germination medium. PGRs, especially auxin, may promote the early development of roots from encapsulated SSEs from which shoots emerge. When plantlets develop both a shoot and a root at an early stage a high percentage of plants are likely to survive and become established.

Conclusion

Artificial oil palm seed production can be undertaken by using SEs or SSEs initiated on both solid and liquid cultures through embryogenesis. In this study the plant material which produced the best results were non-encapsulated SSEs at the coleoptile stage that germinated at a rate of 68.4% and produced complete plantlets at a rate of 57.2% after transfer to a germination medium for 5 months.

The most suitable protocol used for encapsulation was 2.5% sodium alginate polymerized in 100 mM calcium nitrate for 15 minutes. Encapsulated-coleoptile SSEs germinated very fast at an overall rate of 73% after plating on

germination medium for 1 week. The rate of complete plantlets obtained from these SSEs was 9.4% after plating on germination medium for 5 months. The low percentage of complete plantlets obtained from encapsulation was caused by the low concentration of PGR used. However, a high concentration of PGR in all process may result in somaclonal variation.

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References

- Aberlenc-Bertossi, F., Noirot, M. and Duval, Y. (1999). BA enhances the germination of oil palm somatic embryos derived from embryogenic suspension cultures. *Plant Cell, Tissue and Organ Culture* 56: 53-57.
- Aitken-Christie, J., Kozai, T. and Smith, M.A.L. (1995). Automation in plant tissue culture. General introduction and overview. In: *Automation and environmental control in plant tissue culture* (eds. J. Aitken-Christie, T. Kozai and M.A.L. Smith) Kluwer, Dordrecht, pp 1–18.
- Bapat, V.A. and Rao, P.S. (1988). Sandalwood plantlets from “synthetic seeds”. *Plant Cell Reports* 7: 434–436.
- Chehmalee, S. and Te-chato, S. (2008). Induction of somatic embryogenesis and plantlet regeneration from cultured zygotic embryo of oil palm. *Journal of Agricultural Technology*. 4: 137-146.
- Chen, J.T. and Chang, W.C. (2004). Induction of repetitive embryogenesis from seed-derived protocorms of *Phalaenopsis amabilis* var. Formosa Shimadzu. *In Vitro Cellular Developing Biological Plant* 40: 290–293.
- Choi, Y.E., Kim, J.W. and Soh, W.Y. (1997). Somatic embryogenesis and plant regeneration from suspension cultures of *Acanthopanax koreanum* Nakai. *Plant Cell Reports* 17: 84–88.
- de Touchet, B., Duval, Y. and Pannerier, C. (1991). Plant regeneration from embryogenic suspension cultures of oil palm (*Elaeis guineensis* Jacq). *Plant Cell Reports* 10: 529-532.
- das Neves, L.O., Duque, S.R.L., de Almeida, J.S. and Fevereiro, P.S. (1999). Repetitive somatic embryogenesis in *Medicago truncatula* ssp. Narbonensis and *M. truncatula* Gaertn cv. Jemalong. *Plant Cell Reports* 18: 398–405.
- Das, P., Samantaray, S., Roberts, A.V. and Rout, G.R. (1997). *In vitro* somatic embryogenesis of *Dalbergia sissoo* Roxb.—a multipurpose timber-yielding tree. *Plant Cell Reports* 16: 578–582.
- Duval, Y., Durand-Gasselin, T., Konan, K. and Pannetier, C. (1988). *In vitro* vegetative propagation of oil palm (*Elaeis guineensis* Jacq.). *Olagineux* 43: 45-47.
- Ghosh, B. and Sen, S. (1994). Plant regeneration from alginate encapsulated somatic embryos of *Asparagus cooperi* baker. *Plant Cell Reports* 9: 189–194.

- Giridhar, P., Indu, E.P., Ravishankar, G.A. and Chandrasekar, A. (2004). Influence of triacontanol on somatic embryogenesis in *Coffea arabica* L. and *Coffea canephora* P. ex. Fr. *In Vitro Cellular Developing Biological Plant* 40: 200–203.
- Gray, D.J. (1987). Quiescence in monocotyledonous and dicotyledonous somatic embryos induced by dehydration. *HortScience* 22: 810–814.
- Hartmann, H.T., Kester, D.E., Davies, Jr.F.T. and Geneve, R.L. (1997). *Plant Propagation, Principles and Practices*. Sixth edition. Prentice Hall, New Jersey. 130-134.
- Ipekci, Z. and Gozukimizi, N. (2003). Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. *Plant Cell Reports* 22: 16–24.
- Merkle, S.A., Parrott, W.A. and Williams, E.G. (1990). Applications of somatic embryogenesis and embryo cloning. *In Plant tissue culture: applications and limitations* (ed. S.S. Bhojwani) Elsevier, Amsterdam, pp. 67–101.
- Nwankwo, B.A. and Krikorian, A.D. (1983). Morphogenetic potential of embryo and seedling-derived callus of *Elaeis guineensis* Jacq. Var. pisifera Becc. *Annales Botanici Fennici* 51: 65-76.
- Onay, A., Jeffree, C.E. and Yeoman, M.M. (1996). Plant regeneration from encapsulated embryooids and an embryogenic mass of pistachio, *Pistachio vera* L. *Plant Cell Reports* 15: 723–726.
- Paranjothy, K. and Othman, R. (1982). *In vitro* propagation of oil palm. *In Plant Tissue Culture* (Ed. Fugiwara A.). Proceedings 5th International Congress of Plant Tissue and Cell Culture. Tokyo, pp. 747-748.
- Rabechault, H., Ahee, J. and Guenin, G. (1976). Recherches sur la culture in vitro des embryons de palmier a huile (*Elaeis guineensis* Jacq.). XII. Effects de substances de croissance a des doses supraoptimales. Relation avec le brunissement des tissus. *Oleagineux* 31: 159- 163.
- Raemakers, C.J.J.M., Jacobsen, E. and Visser, R.C.F. (1995). Secondary somatic embryogenesis and applications in plant breeding. *Euphytica* 81: 93-107.
- Redenbaugh, K., Slade, D., Viss, P. and Fujii, J.A. (1987). Encapsulation of somatic embryo in synthetic seed coats. *HortScience* 22: 803–809.
- Redenbaugh, K., Fujii, J.A., Slade, D., Viss, P. and Kossler, M. (1991). Artificial seeds-encapsulated embryos. *In: High technology and micropropagation I* (ed. Y.P.S. Bajaj) Biotechnology in agriculture and forestry, vol.17. Springer, Berlin Heidelberg New York, pp. 395–416.
- Rival, A., Berlenc, F.A., Morcillo, F., Tregear, J., Verdeil, J.L. and Duval, Y. (1997). Scaling-up *in vitro* clonal propagation through somatic embryogenesis: the case of oil palm (*Elaeis guineensis* Jacq.). *Plant Cell, Tissue and Organ Culture* 3: 74-83.
- Sanada, M., Sakamoto, Y., Hayashi, M., Mashiko, T., Okamoto, A. and Ohnishi, N. (1993). Celery and lettuce. *In: Synseeds: applications of synthetic seeds to crop improvement* (ed. K. Redenbaugh) CRC Press, Boca Raton, pp 305–327.
- Te-chato, S. and Hilae, A. (2007). High-frequency plant regeneration through secondary somatic embryogenesis in oil palm (*Elaeis guineensis* Jacq. var. tenera). *Journal of Agricultural Technology* 3: 345-357.
- Teixeira, J.B., Sondahl, M.R. and Kirby, E.G. (1993). Somatic embryogenesis from immature zygotic embryos of oil palm. *Plant Cell, Tissue and Organ Culture* 34: 227-233.
- Weissinger, A.K. and Parrott, W.A. (1993). Repetitive somatic embryogenesis and plant recovery in white clover. *Plant Cell Reports* 12: 125–128.

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