Journal of Agricultural Technology 2010 Vol.6(1): 159-170
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Effect of culture media, plant growth regulators and carbon sources on establishment of somatic embryo in suspension culture of oil palm

Kramut, P. and Te-chato, S.*

Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Kramut, P. and Te-chato, S. (2010). Effect of culture media, plant growth regulators and carbon sources on establishment of somatic embryo in suspension culture of oil palm. Journal of Agricultural Technology 6(1): 159-170.

Embryogenic calli were induced from friable embryogenic tissue (FET) on MS medium supplemented with 0.3 mg/l dicamba. These calli had a high proliferation rate at 90% and started to differentiate globular somatic embryos after 1 month of culture. Embryogenic cell suspensions were successfully established using FET on same culture medium and culture condition. The packed cell volume (PCV) of the suspension cell in cultures increased 2 folds after 15 days of culture and number of cell aggregation (more than 10 cells) was 121.68 aggregates/ml. Among auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) at 0.4 mg/l containing MS medium gave the best response on PCV (2.25 ml) and average number of somatic embryos at size of 2-4 mm (20 embryos/flask). Moreover, number of cell aggregation, more than 10 cells, at 106.6 aggregates/ml was obtained in MS medium supplemented with 0.5 mg/l 2,4-D. In case of carbon source, sorbitol (0.2 M) gave the best response on average number of somatic embryo at size of 2 mm (11.33 embryos/flask). This protocol was very benefit to help mass propagation of oil palm plants through cell suspension culture. It would be a key tool for biotechnology in genetic improvement of oil palm as well.

Key words: Somatic embryogenesis, oil palm (*Elaeis guineensis* Jacq.), suspension culture, sorbitol, friable embryogenic tissue (FET)

Introduction

Oil palm is one of the most economically important crops in the world. Cultivation of oil palm has expanded tremendously in recent years such that it is now second only to soybean as a major source of the world supply of oils and fats (Wahid *et al.*, 2004). Interest in palm oil as a biofuel could eventually cause constraints on worldwide supply of edible palm oil and increase the

* Corresponding author: Sompong Te-chato; e-mail: stechato@yahoo.com

pressure for higher yield and/or cultivatable areas (Biofuel, 2007). Processes for the vegetative multiplication of oil palm through somatic embryogenesis have enabled the mass propagation of more than 1 million clonal plantlets to date (Aberlenc-Bertossi *et al*., 1999). Culturing in liquid medium has also been investigated, with the aim of obtaining synthetic seeds on an industrial scale (Gorret *et al*., 2004). Since 1991, two protocols involving embryogenic suspension cultures have been reported for the production of single somatic embryos (de Touchet *et al*., 1991; Teixeira *et al*., 1995), but true to types were limited by high concentration of plant growth regulator. In addition, carbon sources were important role for somatic embryogenesis (Wang *et al*., 1999). Our purposes in these studies were to minimize concentration of auxin and optimize carbon sources for obtaining genetic stability true to type and somatic embryogenesis of oil palm in cell suspension culture.

Materials and methods

Plant material

Embryogenic calli were initiated from high yielding mature oil palm cv. tenera as described by Te-chato and Hilae (2007). Friable embryogenic tissue (FET) was maintained by routine subculture monthly intervals for 2 years on basal Murashige and Skoog (MS) supplemented with 1 mg/l 3,6-dichloro-2-methoxybenzoic acid (dicamba), 200 mg/l Ascorbic acid (As), 3% sucrose and solidified with 0.75% agar-agar. The pH of medium was adjusted to 5.7 prior autoclaving at 1.07 kg/cm^2 at 121^{o}C for 15 min. Cultures were maintained at 28±0.5ºC under 14 h photoperiod at 1,300 lux illumination and subcultured monthly intervals.

Proliferation of FET and its development

FET (approximately 0.1 g) were carefully separated and inoculated on MS medium containing dicamba or 2,4-D at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l. Each concentration of the two auxin containing culture medium was supplemented with 200 mg/l As, 3% sucrose and solidified with 0.75% agar-agar. To determine the most suitable kind and concentration of auxin completely randomize design was employed. Each concentration of the two auxins was done four replicated and each replicated consisted of 5 test tube. After culture under the above condition for one month, fresh weight and a number of somatic embryo at different stage such as, globular embryo (GE), haustorium embryo (HEs) were recorded and statistically compared.

Induction and proliferation of cell suspension culture

For induction of cell suspension, FET at approximately 0.25 gram fresh weight from 1 month-old were transferred to 125 ml Erlenmeyer flask containing 25 ml of liquid medium. The culture media were MS or Y3 medium supplemented with 0.3 mg/l dicamba, 3% sucrose and 200 mg/l As. The cultures were maintained under the same conditions as described earlier, agitation at 110 rpm and subcultured 2 weeks intervals. At two time of subculture (1 month after initiation) packed cell volumn (PCV) and number of cell aggregation cluster in suspension were recorded and statistically compared between the two culture media using least significant difference (LSD).

Effect of plant growth regulators on somatic embryos formation in cell suspension culture

FET cultured in MS liquid medium supplemented 0.3 mg/l dicamba, 3% sucrose and 200 mg/l As for 15 days were transfered to MS liquid medium supplemented with different concentration of ∝-Naphthaleneacetic acid (NAA) 2,4-D or dicamba (0.1 0.2 0.3 0.4 and 0.5 mg/l). The cultures were kept under the same conditions as described earlier. There were 3 replicates, each containing 1 PCV. Observation about PCV and quality of cell suspension were carried out at 3 days intervals for 30 days (from day 0 to 30).

Effect of carbon sources on somatic embryos development

Suspension from MS medium supplemented with 0.3 mg/l dicamba, 3% sucrose and 200 mg/l As were transfered to MS liquid medium supplemented with sucrose and sorbitol two types of carbon sources either 0.2 M sorbitol or 3% sucrose. Under the same conditions as described earlier. There were 3 replicates. Observation about PCV, average number of somatic embryos size (Ø = 2 and 3 mm) per flask after 15 days of cultured. (Ø = diameter)

Data analysis

Data were analysed using ANOVA. Means were separated with Duncan's multiple range tests (DMRT) and least significant difference (LSD) at the 0.05 level. Where, the F-test showed significant differences among means.

Results and discussion

Proliferation of FET and its development

FET (approximately 0.1 g) were inoculated on MS medium containing dicamba or 2,4-D at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l. Each concentration containing 200 mg/l as, 3% sucrose and solidified with 0.75% agar-agar. After 1 month of culture on all treatment gave the different response on the fresh weight (Fig. 1A), number of embryogenic callus (ECs) (Fig. 2A) and haustorium embryos (HEs) (Fig. 2C). For MS medium supplemented with 0.1 mg/l dicamba gave the highest fresh weight which contained many globular structure and haustorium stage (Figs. 1A, B). Callus was gradually increased by increasing time of culture. After 1 month of culture, MS medium supplemented with 0.3 mg/l dicamba gave the best response on number of ECs (Fig. 1C). Contrary result was obtained by Chehmalee and Te-chato (2008) in zygotic embryo culture where embryogenic callus proliferation was achieved on MS medium supplemented with 0.5 mg/l dicamba. This might be due to the different sources of FET. The previous work used zygotic embryo derived FET while this present study used young leaf-derived FET. Moreover, period of culture were quite different. FET from leaf exposed to auxin for more than two years. So, a low concentration of dicamba required for proliferation of cell suspension. Dicamba was found to be the best auxin for *in vitro* mass propagation of both seedling and young leaves of both mature oil palm (Te-chato *et al*., 2003). In addition, embryoids developed on medium containing 0.1 mg/l dicamba was found to be superior in inducing early stage of embryoid subsequent to further development of mature or haustorium embryoids (Te-chato, 1998). Decrease in concentration of dicamba stimulated proliferation rate of EC and also promoted a large number of embryoid formation whereas 2,4-D produced phenolic compound in MS medium (Fig. 2B). Many authors reported the effect of 2,4-D on phenolic compound production from plants tissue culture (Davies, 1972; Zaid, 1987; de-Touchet *et al*., 1991; Kanchanapoom and Tinnongjig, 2001). Our results support similar observations made in oil palm tissue culture. Moreover, FET had the smallest size of globular structure came out from both peripheral and sub-peripheral cells. The result supported that dicamba promoted cells more than one layer to produce nodular structures whereas 2,4-D promoted only one layer like the report of Chehmalee and Te-chato (2007).

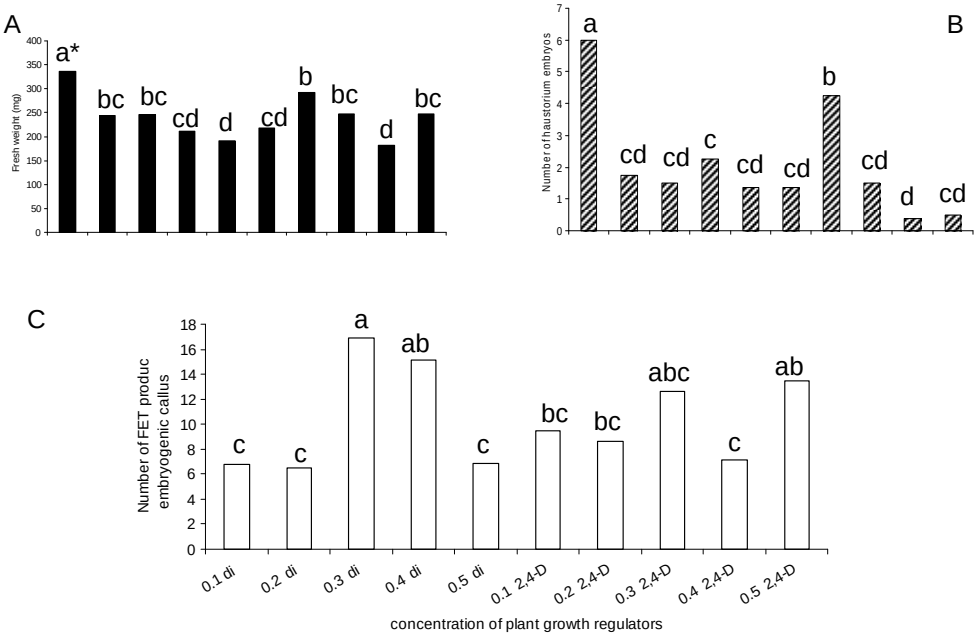

Fig. 1. Effect of various concentrations of dicamba or 2,4-D (0.1-0.5 mg/l) after culturing FET on MS medium in the presence of 200 mg/l As for 1 month. A: Fresh weight B: Number of haustorium embryos C: Number of FET produced embryogenic callus.

* Value followed by different letter in term of type of PGR and concentrations are significantly different according to DMRT-test at P<0.05 level.

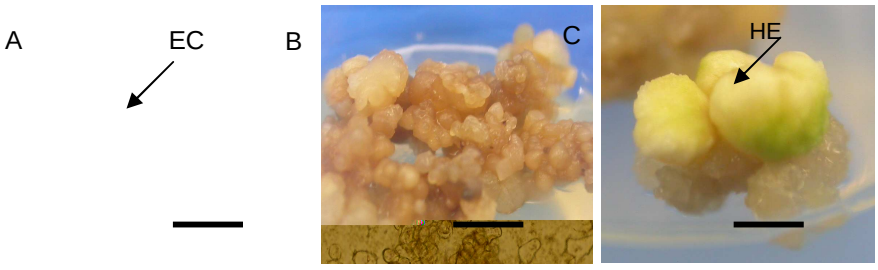

Fig. 2. Different types of callus obtained from various concentrations of plant growth regulators on MS media after one month of culture. A: EC in FET cultured on 0.3 mg/l dicamba containing medium (bar: 3 mm) B: NC in FET cultured on 0.5 mg/l 2,4-D containing medium (bar: 3 mm) C: HE in FET cultured on 0.1 mg/l dicamba containing medium (bar: 1 mm).

Induction and proliferation of cell suspension culture

Successful on the induction of cell suspension is known to depend on type of culture medium used. Therefore, two basal media, MS and Y3, generally employed in tissue culture of oil palm were examined. Those culture media were supplemented with 0.3 mg/l dicamba, 200 mg/l As and 3% sucrose. Both culture media gave the same results in somatic embryo sizes which were classified into three sizes; less than 1 mm (12%), 1 to 2 mm (83%) and larger than 2 mm (5%) (data not shown). MS medium gave the higher PCV than Y3 medium During the maintenance phase a rapid growth of cell suspension was observed in this culture medium. After culture for 24 days, average growth rate increased 2 folds at each time of sub-culture (15 days intervals) (Fig. 3). Thiruvengadam et al. (2006) reported that key element for the induction of somatic embryogenesis was the presence of high levels of nitrogen in the form of oganic compound that enhance embryo initiation and maturation. In comparison between MS and Y3 medium, MS found to have a higher level of nitrogen than Y3 lead to the better proliferation rate of PCV and embryo differentiation as well. Our results suggested that the high organic compound of the MS medium might contributory towards enhancement of somatic embryogenesis. However, Teixeira et al. (1995) reported that Y3 medium gave the better response in embryogenesis of different cultivar of oil palm. The different results might be due to source of explants. Moreover, culture medium composition also affected on number of cell aggregate in suspension culture (Table 1). MS medium gave the higher number of cell aggregate (more than 10 cells). However, oxidation and phenolic compound production occurred at day 15 and 21 after culture in MS and Y3 medium, respectively. These might be showed the effect of medium component, especially amino acid might be caused highly production of phenolic compound after culture for a long period (more than 7 days). Similar result was observed by Zaid (1987) in date palm.

Table 1. Effect of culture media supplemented with 0.3 mg/l dicamba, 200 mg/l as, and 3% sucrose on number of cell aggregates in suspension culture (25 ml liquid medium) at 15 days of culture.

Culture media	Number of cell aggregate		
	< 5	5-10	> 10
MS	1.675	55	121.68
Y3	0	45	50
$LSD_{.05}$	0.18	1.73	3.13
C.V. (%)	244.95	38.08	40.22

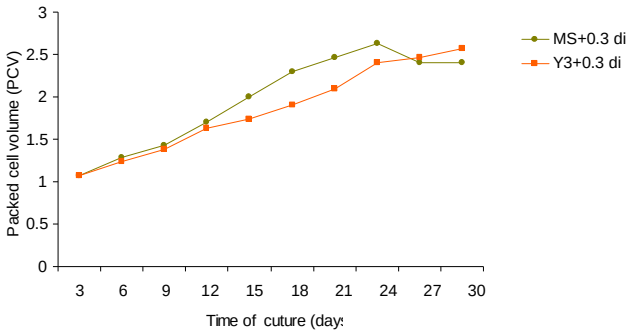

Fig. 3. Effect of culture media on PCV after culturing cell suspension in MS medium or Y3 medium supplemented with 0.3 mg/l dicamba and 200 mg/l As for 1 month.

Effect of plant growth regulators on growth and development of somatic embryos in suspension culture

Suspension culture using MS medium supplemented with 0.3 mg/l dicamba, 3% sucrose and 200 mg/l as subsequent mechanical sieving with stainless-steel sieves at pore size of 1 mm allowed rapid production of fine suspensions made of small size aggregates. All of the auxins were shown capable of initiation cell suspensions and embryo development. However, MS medium supplemented with 0.4 mg/l 2,4-D produced the greater PCV (2.25 ml) in comparison with the other treatment after 15 days of culture (Fig. 4). In case of number of somatic embryos (size: 2-4 mm), 2,4-D at the same concentration promoted the best result as well. The average number of somatic embryos obtained in 0.4 mg/l 2,4-D containing medium was 20 embryos/flask (Fig. 5). Similar result was obtained in cell suspension culture of Momordica charantia L.. Increase in concentration of 2,4-D stimulated proliferation rate of cell in suspension and also promoted globular stage somatic embryo (Thiruvengadamet al., 2006). Jimenez and Thomas (2005) reported that among individual groups of auxin, 2,4-D promoted the transition from pro-embryonic mass to somatic embryos. However, high concentration of 2,4-D was reported to produce phenolic compounds in culture medium (Zaid, 1987; de Touchet et al., 1991). This result was contrary to the report of Tefchato et al. (2008) in oil palm. They suggested that dicamba was an important plant growth regulator in embryogenesis of oil palm either alone or in combination with BA or KN. Furthermore, number of cell aggregate in MS medium supplemented with 0.5 mg/l 2,4-D gave the best result after 15 days of culture (Fig. 6). Both cell aggregate might be initiated to embryoid or somatic embryo.

This report described two types of cell aggregate, 5 to 10 cells and more than 10 cells. For aggregate consisted of more than 10 cells, those cell showed dense cytoplasm (Fig. 7B) whereas 5 to 10 cell aggregate consisted of large vacuolar cells (Fig. 7A). de Touchet *et al.* (1991) reported that embryogenic cells were dividing actively and expressed a round prominent nucleus, and a dense cytoplasm with small vacuoles.

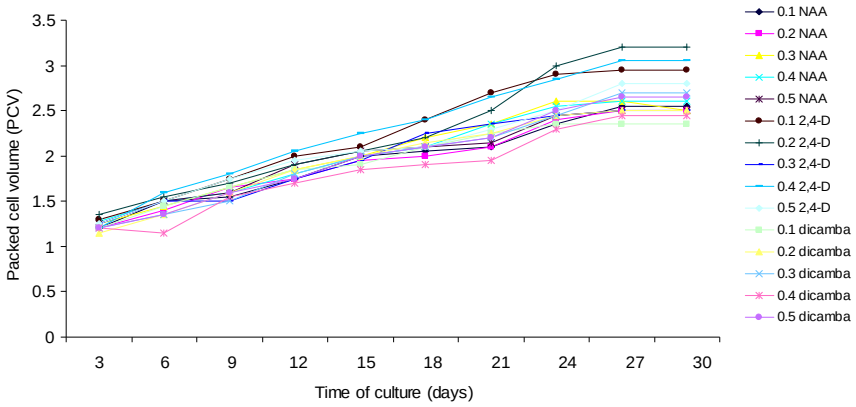

Fig. 4. Effect of plant growth regulators on packed cell volume after culturing somatic embryo suspension on MS medium supplemented with different concentration of auxin and 200 mg/l As for 1 month.

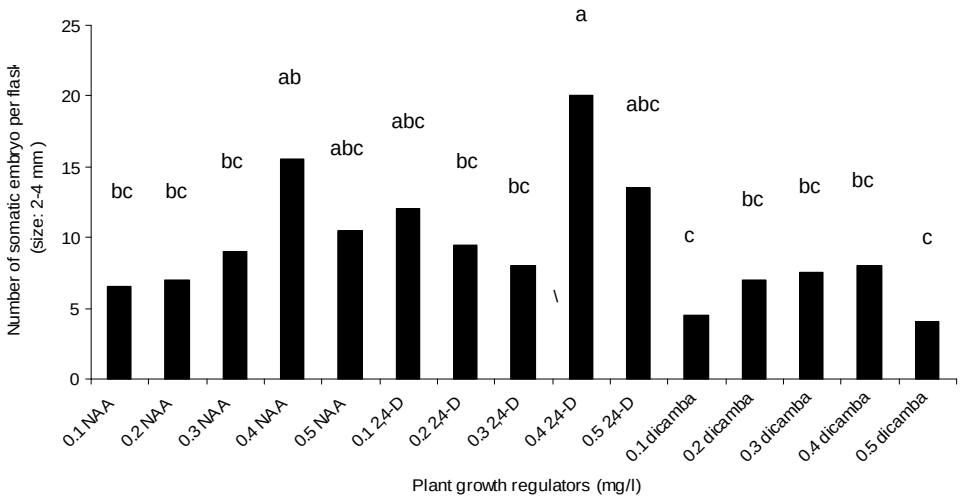

Fig. 5. Effect of plant growth regulators on number of somatic embryos (size: 2-4 mm) in MS medium supplemented with different concentration of auxin and 200 mg/l As after culture for 15 days.

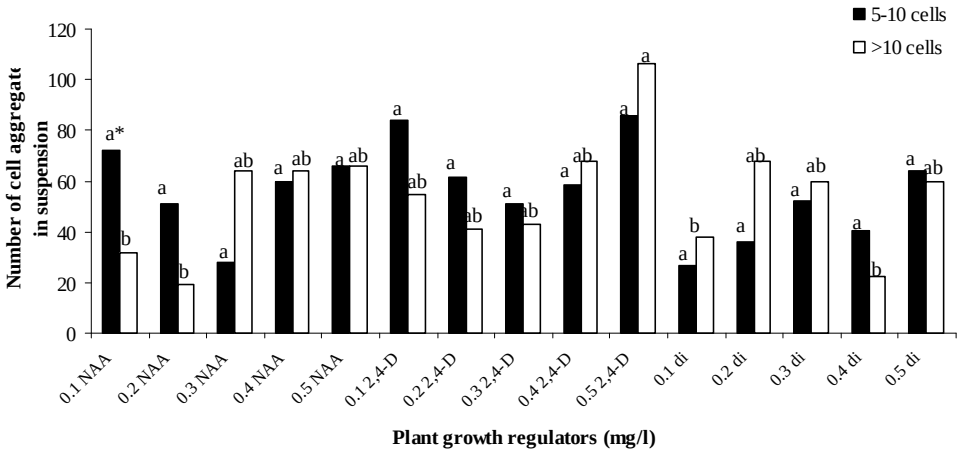

Fig. 6. Effect of plant growth regulators supplemented with 200 mg/l As, and 3% sucrose on number of clusters of cells aggregation (25 ml liquid medium) at 15 days of culture.
* Value followed by different letter in term of type of PGRs in the same colour are significantly different according to DMRT at P<0.05 level.

A **B**

Fig. 7. Clusters of cells aggregation in MS medium supplemented with 0.5 mg/l 2, 4-D after 15 days of culture**.** A: 5 to 10 cells, B: more than 10 cells (Bar: 50 µm**).**

Effect of carbon source on somatic embryo development

FET in liquid MS medium was supplemented with 0.3 mg/l dicamba and 200 mg/l as and replace sucrose with 0.2 M sorbitol produced 2 mm somatic embryos in size after 15 days of culture (Fig. 8) more than 3% sucrose (Table 2).

Similar result was obtained in culturing friable calli of sweet potato and sorbitol also play important role in cell growth and development (Wang *et al.*, 1999). In addition, sorbitol was also reported to induce secondary somatic embryos from haustorium embryo (HE) culture of oil palm (Chehmalee and Te-chato, 2008). Full-strength MS medium supplemented with 0.2 M sorbitol produced significantly higher percentage and number of SSEs (Te-chato and Hilae, 2007). In culture medium supplemented with 3% sucrose, embryos swelled and tended to develop into haustorium-like structures. Similar result was obtained in culturing nodular calli of oil palm (de-Touchet *et al*., 1991). Sorbitol act as osmotic agent caused a change in protein level in the cell. One of that protein involved in embryogenesis leading to further development of somatic.

Table 2. Effect of carbon source on number of somatic embryos size/flask after 15 days of culture.

Carbon source	Number of somatic embryos at size of (mm)	
	2	3
sucrose	2.67b	3.33a
sorbitol	11.33a	1.67a
$LSD_{.05}$	6.34	4.72
C.V. (%)	39.98	83.27

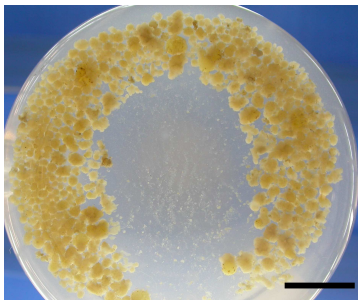

Fig. 8. Somatic embryo size in MS medium supplemented with 0.2 M sorbitol and 200 mg/l As after 15 days of culture. (Bar: 6 mm).

Conclusion

The present study successfully describes somatic embryogenesis from culturing FET in liquid MS medium suspension culture. The highest weight (33.6 mg) and number of HEs (6) were obtained in MS medium supplemented with 0.1 mg/l dicamba while the highest number of EC (16.88) was cultured on MS medium supplemented with 0.3 mg/l dicamba. MS liquid medium

supplemented with 0.3 mg/l dicamba gave the best response on PCV (2 ml) and number of cell aggregate at size of more than 10 cells (121.68 aggregates/ml) after 15 days of culture. MS medium supplemented with 0.4 mg/l 2, 4-D gave the best response on PCV (2.25 ml PCV) and the average number of somatic embryos at size of 2-4 mm (20 embryos/flask) in suspension culture while the highest number of cell aggregate at size of more than 10 cells (106.6 aggregates/ml) were cultured in MS medium supplemented with 0.5 mg/l 2,4-D after 15 days of culture. Somatic embryos about 2 mm (11.33 embryos /flask) were cultured in MS medium supplemented with 0.2 M sorbitol.

Acknowledgments

The authors are grateful to the Faculty of Natural Resources and the Graduate School of Prince of Songkla University. This research is partially supported by the Center for Agricultural Biotechnology, Postgraduate Education and Research Development Office, Commission on Higher Education.

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.

Biofuel. (2007). Journey to forever-how to make your own clean burning biofuel, biodisel from cooking oil, fuel alcohol, renewable energy, glycine, soap making. [Online] Available http://journeytoforever.org/biofuel.html (access on 12 June 2007).

Chehmalee, S. and Te-chato, S. (2007). Genotypes, physiological ages of zygotic embryos and auxin as affect on germination and callus formation of oil palm. International Conference on Integration of Science and Technology for Sustainable Development, Bangkok, Thailand. 26-27 April 2007. pp. 35-39.

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.

Davies, M.E. (1972). Polyphenol synthesis in cell suspension cultures of Paul's Scarlet Rose. Planta 104: 50-65.

De Touchet, B., Duval, Y. and Pannetier, C. (1991). Plant regeneration from embryogenic suspension culture of oil palm (*Elaeis guineensis* Jacq). Plant Cell Reports 10: 529-532.

Gorret, N., Rosli, S.K., Oppenheim, S.F., Willis, L.B., Lessard, P.A., Rha, C. and Sinskey, A. J. (2004). Bioreactor culture of oil palm (*Elaeis guineensis*) and effects of nitrogen source, inoculum size, and conditioned medium on biomass production. Journal of Biotechnology 108: 253-263.

Jimenez, V.M. and Thomas, C. (2005). Participation of plant hormones in determination and progression of somatic embryogenesis. Plant Cell Monographs 2: 104-118.

Kanchanapoom, K. and Tinnongjig, S. (2001). Histology of embryoid development in oil palm (Elaeis guineensis Jacq.) cell suspension culture. Songklanakarin Journal of Science and Technology 23: 643-648.

Koscielniak, J.B., Koscielniak, J., Filek, M. and Janeczko, A. (2008). Rapid production of wheat cell suspension cultures directly from immature embryos. Plant Cell, Tissue and Organ Culture 94: 139–147.

Te-chato, S. (1998). Callus induction from cultured zygotic embryo of oil palm subsequent to plantlet regeneration. Songklanakarin Journal of Science and Technology 20: 1-6.

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.

Te-chato, S. Hilae, A. and In-peuy, K. (2008). Effects of cytokinin types and concentrations on growth and development of cell suspension culture of oil palm. Journal of Agricultural Technology 4: 157-163.

Te-chato, S., Hilae, A. and Yeedum, I. (2003). Histological study on oil palm of somatic embryos development as affected by sources of leaf explants and auxin. Journal of Agricultural Science 36: 243-250.

Teixeira, J.B., Sondhal, N.R., Nakamura, T. and Kirby, E.G. (1995). Establishment of oil palm cell suspensions and plant regeneration. Plant Cell, Tissue and Organ Culture 40: 105–111.

Thiruvengadam, M., Mohamed, V.S., Yang, V.H. and Jayabalan, N. (2006). Development of an embryogenic suspension culture of bitter melon (*Momordica charantia* L.). Scientia Horticulturae 109; 123-129.

Wahid, M.B., Abdullah, S.N.A. and Henson, I.E. (2004). Oil palm-achievements and potential.Proceeding of the 4th International Crop Science Congress, Brisbane, Australia. 26 Sep-1 Oct 2004. pp. 1-13.

Wang, H.C., Chen, J.T. and Chang, W.C. (2006). Somatic embryogenesis and plant regeneration from leaf, root and stem-derived callus cultures of *Areca catechu.* Biologia Plantarum 50: 279-282.

Wang, H.L., Lee, P.D., Liu, L.F. and Su, J.C. (1999). Effect of sorbitol induced osmotic stress on the changes of carbohydrate and free amino acid pools in sweet potato cell suspension cultures. Botanical Bulletin of Academia Sinica 40: 219-225.

Zaid, A. (1987). Invitro browning of tissues and media with special emphasis to date palm cultures. Acta Horticulturae 212: 561-566.

(Received 19 February 2009; accepted 3 October 2009)