Initiation of Callogenesis from Unfertilized Ovary Explants of Oil Palm (*Elaeis guineensis* Jacq.)

H.M.D.M.P. Herath, L.M.H.R Alwis
Uva Wellassa University, Badulla, Sri Lanka

and

H.D.D. Bandhupriya
Coconut Research Institute, Lunuwila, Sri Lanka

Introduction

Oil palm is an efficient oil producing crop which has high economic value. Due to less capability of vegetative propagation, tissue culture is considered to be the only approach to produce clonal plantlets for oil palm. Several research based on somatic embryogenesis have been undertaken using various tissues (Euewens, 1976; Guedes et al., 2011) but no research work on oil palm ovary explants is reported. The objectives of this study were to find out relationship between maturity stages of inflorescence for callogenesis, to identify suitable *in vitro* culture media for callogenesis, and to determine the optimum 2, 4-D concentration for callus induction and multiplication in same callusing media.

Methodology

Unfertilized ovaries obtained from immature female inflorescences of oil palm (*Elaeis guineensis*Jacq.) were tested as a source of explants for callogenesis. Inflorescence maturity stages of -2, -3 and -4 were cultured on Y3 (Eeuwens, 1976), CRI-72 (Karunarathne and Periypperuma, 1989) and OPC3 medium (Karun and Sajini, 1996) supplemented with 2,4-D levels of 160 µM, 180 µM and 200 µM. Collected female flowers were disinfected wit h 5% commercial bleach (chlorox) for 10 minutes followed by five rinses with sterile water. Ovaries were dissected out and cultured in vials containing 10 ml of callus induction medium under sterilized condition. Ovary cultures were incubated under dark condition at 28 ^{0}C in an incubation room until callus proliferation. Cultured ovaries were sub-cultured in to fresh medium (same callusing medium) and callus multiplication possibility was tested. Complete Randomized Design (CRD) with three factor factorial analysis was used as the experimental design.

Results and Discussion

Development of callus was visible during the seven weeks after culture establishment. Callus proliferation occurred when sub-cultured in to the same medium. Callus initiation of immature oil palm ovaries of different inflorescence stages tested indicated no significant difference (Table 1).

Table 1. The effect of inflorescence maturity stages on percentage callus production in oil palm ovaries

Percentage callus production in oil palm ovaries			
Maturity stage of ovary [a]	160[b]	180[b]	200[b]
-2	4	5	0
-3	3	9	0
-4	0	0	9

[a]Maturity of ovaries decrease from -2 to -4 stage (i.e. the most immature stage is -4). [b] 2,4-D concentrations (µM). (Pr>ChiSq) < 0.05; significant

Guedes et al. (2011) found that the percentage of embryogenic explants in a culture medium with 225 µM of 2,4-D gave the best results irrespective of the position of explants in the rachillae. Although the present study revealed that there is no significant difference ($p<0.05$) among oil palm inflorescence maturity stages, Perera et al. (2007) verified the percentage callus production in unfertilized coconut ovaries of -4 maturity stage with 100 µM 2,4-D was significantly ($p<0.05$) higher (30%) than all other treatments tested.

Table 2. The effect of different media compositions and different 2,4-D concentrations on percentage callus production in oil palm ovaries at different stages of maturity.

Maturity stage of Ovary [a]	Y3 [b]			CRI 72 [b]			OPC3 [b]		
	160 [c]	180 [c]	200 [c]	160 [c]	180 [c]	200 [c]	160 [c]	180 [c]	200 [c]
-2	0	0	0	4	5	0	0	0	0
-3	6	2	0	3	9	9	0	0	0
-4	0	0	0	0	0	9	0	0	0

[a]Maturity of ovaries decrease from -2 to -4 stage (i.e. the most immature stage is -4). Media [b] compositions ($g.l^{-1}$). [c]2,4-D concentrations (µM). (Pr>ChiSq) < 0.05; significant

Callus initiation of immature oil palm ovaries with different culture media and different 2,4 -D concentrations tested indicated no significant difference ($p<0.05$) and also there was no interaction effect between callus induction media and 2,4-D concentrations. These results are in agreement with those of Thuzar et al. (2011) who found that the effect of media on embryogenic callus induction using oil palm zygotic embryos was not significantly different and interaction effect between callus induction media and auxins was not significantly different. However, Teixeira *et al*. (1994) found that after five months of culture, somatic embryos induction of oil palm using immature inflorescence of the *pisifera* variety occurred at a concentration of 500 µM of 2,4-D. According to Perera et al. (2007), coconut ovary explants give rise to 41% callusing when cultured in a medium containing 100 µM of 2,4-D and Thuzar et al. (2011) found that 2,4-D gives the significantly highest callus induction rate (79.8%).

Conclusions

This study indicated that the callogenesis of unfertilized oil palm ovaries are not significantly affected by the inflorescence maturity stage, culture medium and 2,4-D concentrations tested. In conclusion, callus initiation can be achieved using the procedure described here. Callus tissues of oil palm ovaries can be established and grown in culture. Callus can be sub -cultured in the same callusing medium and it is possible to multiply the callus.

References

Eeuwens, C.J., 1976. Mineral requirement for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera)* and cultured *in vitro*. Physiologia Plantarum, 36(1), 23-28.

Guedes, R.S., Silva, T.L., Luis, Z.G., Scherwinski-Pereira, J.E., 2011. Initial requirements for embryogenic calluses initiation in thin cell layers explants from immature female oil palm inflorescences. African Journal of Biotechnology, 10(52), 10774-10780.

Karun, A., Sajini, K.K., 1996. Plant regeneration from leaf explants of oil palm seedlings. Current Science, 71, 922-926.

Karunaratne, S., Periyapperuma, K., 1989. Culture of immature embryos of coconut (*Cocosnucifera L.*) callus proliferation and somatic embryogenesis. Plant Science, 62, 247-253.

Perera, P.I.P., Hocher, V., Verdeil, J.L., Doulbeau, S., Yakadawala, D.M.D., Weerakoon, L.K., 2006. Unfertilized ovary: a novel explants for coconut (*Cocos nucifera* L.) somatic embryogenesis. Plant cell Rep, 26, 21-28.

Teixeira, J.B., Sondahl, M.R., Kirby, E.G., 1994. Somatic embryogenesis from immature inflorescences of oil palm. Plant Cell Rep, 13(34), 247-250.

Thuzar, M., Vanavichil, A., Tragoonrung, S., Jantasuriyarat, C., 2010. Efficient and rapid plant regeneration of oil palm zygotic embryos cv.'Tenera' through somatic embryogenesis. Acta Physiologiae Plantarum, 33(1), 123-128.