Appendix 1

Composition of Murashige and Skoog (MS) medium (1962).

Macroelements

Ammonium nitrate, NH_4NO_3	1,650 mg
Potassium nitrate, KNO_3	1,900 mg
Calcium chloride, $CaCl_2 . 2H_20$	440 mg
Potassium dihydrogen phosphate, KH_2PO_4	170 mg
Magnesium sulfate, $MgSO_4 . 7H_2O$	370 mg

Chelated iron

Na_2-EDTA . $2H_2O$	37.3 mg
Ferrous sulfate, $FeSO_4 . 7H_2O$	27.8 mg

Microelements

Boric acid, H_3BO_3	6.2 mg
Manganese sulfate, $MnSO_4 . 4H_2O$	16.9 mg
Zinc sulfate, $ZnSO_4 . 7H_2O$	6.14 mg
Potassium iodine, KI	0.83 mg
Sodium molybdate, $Na_2MoO_4 . 2H_2O$	0.25 mg
Copper sulfate, $CuSO_4 . 5H_2O$	0.025 mg
Cobaltous chloride, $CoCl_2 . 6H_2O$	0.025 mg

Appendix 1 (Continued)

Growth factor	
Myo-inositol	100 mg
Amino acid	
Glycine	2 mg
Vitamins	
Thiamine HCl	0.1 mg
Nicotinic acid	0.5 mg
Pyridoxin HCl	0.5 mg

Appendix 2

Composition of Eeuwens (Y_3) medium (1976).

Macroelements

Potassium nitrate, KNO_3	2,020 mg
Potassium chloride, KCl	1,492 mg
Ammonium chloride, NH_4Cl	535 mg
Sodium dihydrogen phosphate, $NaH_2PO_4 . 2H_2O$	312 mg
Calcium chloride, $CaCl_2 . 2H_2O$	294 mg
Magnesium sulfate, $MgSO_4 . 7H_2O$	247 mg

Chelated iron

Na_2-EDTA . $2H_2O$	37.3 mg
Ferrous sulfate, $FeSO_4 . 7H_2O$	13.9 mg

Microelements

Manganese sulfate, $MnSO_4 . 4H_2O$	11.2 mg
Potassium iodine, KI	8.3 mg
Zinc sulfate, $ZnSO_4 . 7H_2O$	7.2 mg
Boric acid, H_3BO_3	3.1 mg
Cobaltous chloride, $CoCl_2 . 6H_2O$	0.24 mg
Sodium molybdate, $Na_2MoO_4 . 2H_2O$	0.24 mg

Appendix 2 (Continued)

Copper sulfate, $CuSO_4 . 5H_2O$	0.16 mg
Nickle chloride, $NiCl_2 . 6H_2O$	0.024 mg

Growth factor

Myo-inositol	100 mg

Vitamins

Thiamine HCl	0.5 mg
Nicotinic acid	0.05 mg
Pyridoxin HCl	0.05 mg
Biotin	0.05 mg
Calcium pantothenate	0.05 mg

Appendix 3

Composition of CPW salts solution (Power *et al.*, 1984) in 1000 ml.

Potassium dihydrogen phosphate, KH_2PO_4	27.2 mg
Potassium nitrate, KNO_3	101.0 mg
Calcium chloride, $CaCl_2 . 2H_2O$	1,480.0 mg
Manganese sulfate, $MgSO_4 . 4H_2O$	246.0 mg
Potassium iodine, KI	0.16 mg
Copper sulfate, $CuSO_4 . 5H_2O$	0.025 mg
2-N-morpholinoethanesulphonic acid, MES	6,000.0 mg

Appendix 4

Protocol for determination of protoplast viability (FDA stain) (Widholm, 1972).

1. Prepare a stock solution of FDA (1-5 mg/ml acetone) and store at 0^0 C in the dark.
2. Add 1 drop of the stock solution to 10 ml CPW salts solution.
3. Mix equal volumes of the FDA solution with a dense protoplast suspension in culture medium.
4. After 5 min examine by fluorescence microscopy, pipette a sample onto a hemacytometer, and count the fluorescent protoplasts on an Olympus inverted microscope (IMT-2) with an UV light source.

$$\%\,protoplast\quad viability = \frac{no.\ \ of\ \ fluorescing\ \ protoplasts}{total\ \ no.\ \ of\ \ protoplasts} \times 100$$

Appendix 5

Protocol for preparation of oil palm embryo protoplasts.

1. Add the macerated embryos approximately 0.2 g to 5 ml of CPW protoplast enzyme solution, 0.6 M sorbitol. Divide the mixture into 60 ×15 mm cell culture disk.
2. Place protoplast disks on stationary condition at 25° C for 48 h, in the dark.
3. Filter the digestion mixture sequentially though 180, 74 and 42 μm nylon mesh filters. Centrifuge the filtrate at 52 g for 10 min and separate off the supernatant.
4. Resuspend the protoplasts pellet in approximately 5 ml of CPW sucrose 0.6 M and then add on top with further sorbitol CPW 0.6 M to 15 ml centrifuge tube. Re-sediment the protoplasts as above but extend time to 12 min.
5. Harvest the purified protoplasts and wash them with washing medium containing MS salts solution and sorbitol 0.5 M. After the second wash resuspend the pellet in 15 ml corning centrifuge tube to 1 ml.
6. To determine viable protoplasts. *See* appendix 4.
7. Adjust the concentration of the source suspension to 2 x 10^5 protoplasts/ml by resuspend them in the appropriate volume of 0.4 M culture medium.

Appendix 6

Protocol for flow cytometric analysis (Pfosser *et al.*, 1998).

1. Chop a small amount of plant material (typically 20 mg) with a new razor blade or a sharp scalpel in 1ml of ice-cold Tris-$MgCl_2$ buffer in a Petri dish. It is preferable to include a DNA fluorochrome (Propidium iodide) in the buffer prior to chopping. Alternatively, this compound may be added immediately after the filtration (step 2). The stains are used in the following concentrations: 50 µg/ml propidium iodide + 50μg/ml RNase.

   The actual quantity of plant material to be used for nuclei isolation depends both on the type of tissue and on the species, and must be determined experimentally (higher quantities are usually needed of callus or cultured cells).
2. Filter the suspension through a 42 μm nylon mesh.
3. Store on ice prior to analysis (a few minutes to one hour).
4. Analyse relative DNA content of isolated nuclei.

Appendix 6 (Continued)

Propidium iodide stock solution

1 mg/ml propidium iodide	50 mg

- Dissolve in 50 ml H_2O
- Filter through a 0.22 µm filter to remove small particles
- Store at -20°C in 0.5 ml aliquots

RNase stock solution

1 mg/ml RNase (IIA Sigma)	25 mg

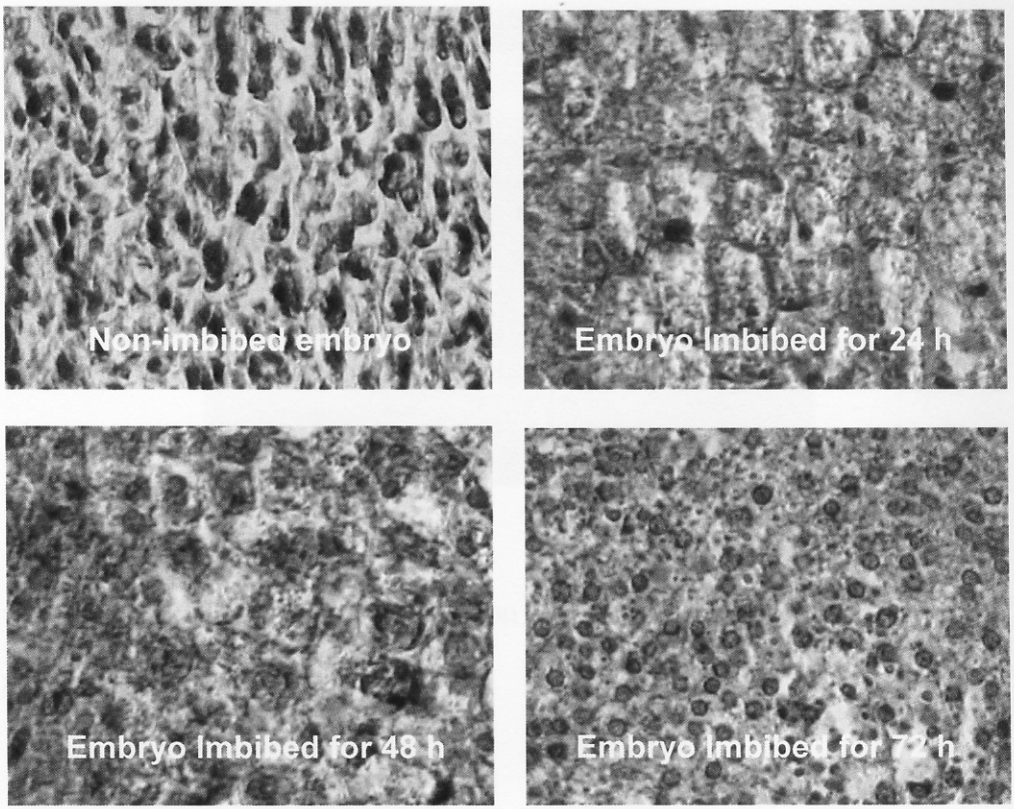
- Dissolve in 25 ml H_2O
- Filter through a 0.22 µm filter to remove small particles
- Heat to 90°C for 15 min to inactivate DNases
- Store at -20°C in 0.5 ml aliquots

Tris-$MgCl_2$ buffer stock solution

0.2 M Tris	4.84 g
4 mM $MgCl_2$. 6H2O	162.64 mg
0.5% Triton X-100	1 ml

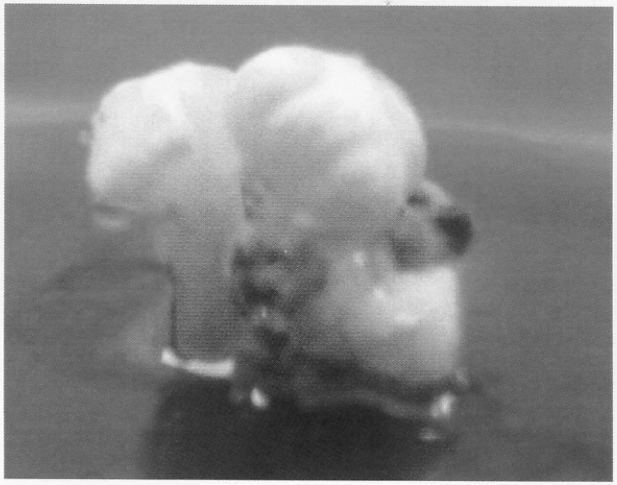
- Adjust volume to 200 ml
- Adjust pH to 7.5
- Filter through a 0.22 μm filter; store at 4°C

Appendix 7



Lipid grains and storage proteins in oil palm embryos after imbibed for 24-72 h.

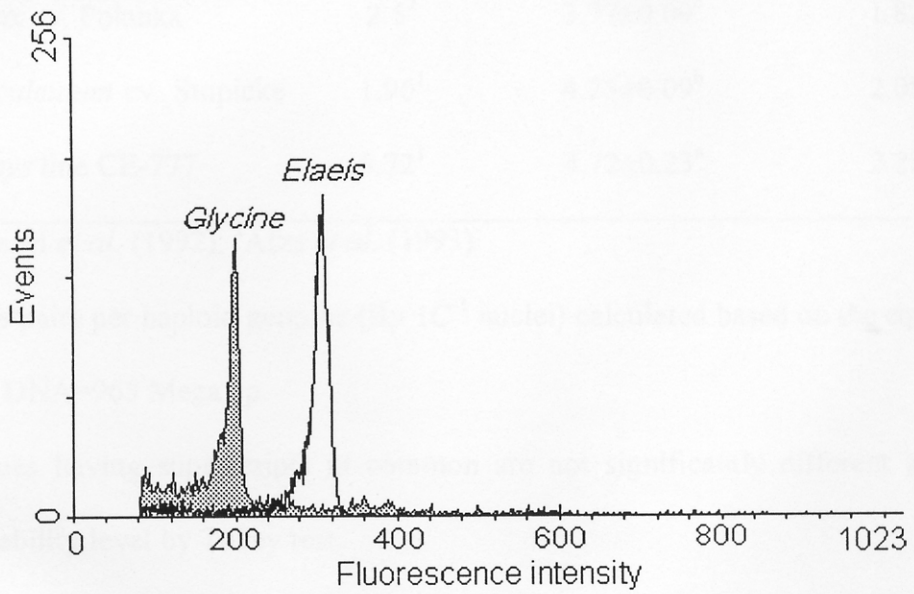
Appendix 8



Embryogenic calli of oil palm cultured on MS medium supplemented with 4.5 μM 2,4-D and 0.1% activated charcoal.

Appendix 9

The results of flow cytometric analysis of oil palm using reference plant as an external standard:



Histogram of fluorescence intensity of nuclei isolated from oil palm using *Glycine max* cv. Polanka as an external reference plant.

Appendix 9 (Continued)

Genome size (2C nuclear DNA content in pg) of *E. guineensis* Jacq. compared with various reference plants. The means are based on 10 replicate experiments.

Reference plants	DNA content (pg $2C^{-1}$) (Mean±SE)*		
	Reference plants	*Elaeis* (external reference)*	*Elaeis* (Base pairs)[3]
G. max cv. Polanka	2.5[2]	3.77±0.09[c]	1.82×10^9
L. esculentum cv. Stupicke	1.96[1]	4.25±0.09[b]	2.05×10^9
Z. mays line CE-777	5.72[1]	4.72±0.23[a]	2.28×10^9

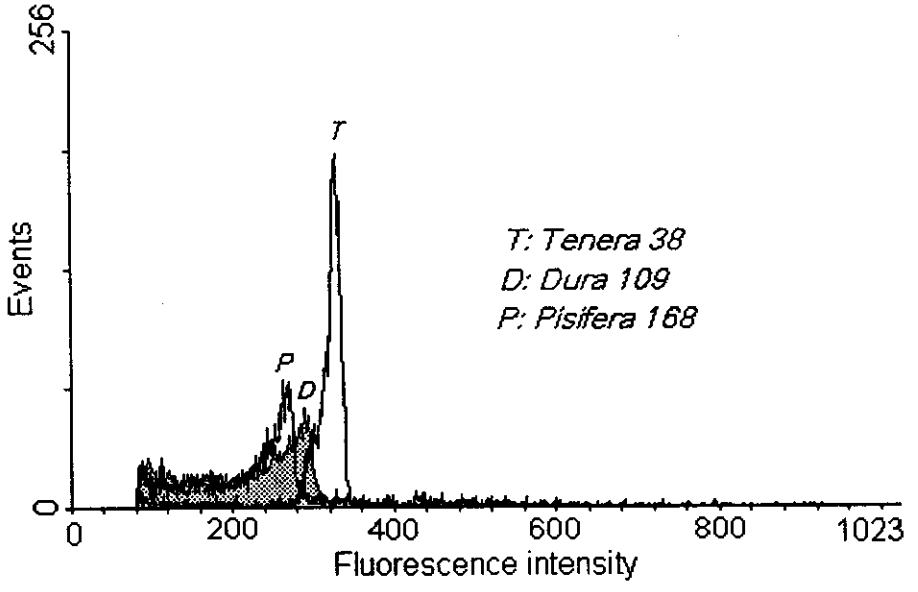
[1]Dolezel *et al.* (1992); [2]Afza *et al.* (1993)

[3]Base pairs per haploid genome (Bp $1C^{-1}$ nuclei) calculated based on the equivalent of 1 pg DNA=965 Mega bp.

*Values having superscripts in common are not significantly different at the 0.05 probability level by Tukey test.

Appendix 9 (Continued)

Cultivars comparison of oil palm:



Comparison of fluorescence intensity histogram among *E. guineensis* Jacq. cv. *Dura* (D109), *Pisifera* (P168) and *Tenera* (T38) using *Glycine max* cv. Polanka as an external reference plant.

Appendix 9 (Continued)

Genome size (2C nuclear DNA content in pg) of *E. guineensis* Jacq. cv. *Dura*, *Pisifera* and *Tenera* using *Glycine max* cv. Polanka as an external standard. The means are based on 5 replicate experiments.

Elaeis cultivars	DNA content (pg $2C^{-1}$) (Mean±SE)*	*Elaeis* (Base pairs)[2]
G. max cv. Polanka	2.5[1]	1.21×10^9
Dura (D109)	3.46 ± 0.02^b	1.67×10^9
Pisifera (P168)	3.24 ± 0.01^c	1.56×10^9
Tenera (T38)	3.76 ± 0.02^a	1.81×10^9

[1]Afza *et al.* (1993)

[2]Base pairs per haploid genome (Bp $1C^{-1}$ nuclei) calculated based on the equivalent of 1 pg DNA=965 Mega bp.

*Values having superscripts in common are not significantly different at the 0.05 probability level by Tukey test.

Publications

Srisawat T. and Kanchanapoom K. 2005. The influence of physical conditions on embryo and protoplast culture in oil palm (*Elaeis guineensis* Jacq.). *ScienceAsia*. 31: 23-28.

Srisawat T., Kanchanapoom K., Pattanapanyasat K. Srikul S. and Chuthammathat W. 2005. Flow cytometric analysis of oil palm: a preliminary analysis for cultivar and genomic DNA alteration. *Songklanakarin J. Sci. Technol.* in press.

Srisawat T, Kanchanapoom K and Mii M. 2005. Isolation, culture and cell colony formation of protoplasts from embryos of oil palm (*Elaeis guineensis* Jacq.). (manuscript).