Appendix 1

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

Macroelements

Ammonium nitrate, $\text{NH}_4\text{NO}_3$ 1,650 mg
Potassium nitrate, $\text{KNO}_3$ 1,900 mg
Calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 440 mg
Potassium dihydrogen phosphate, $\text{KH}_2\text{PO}_4$ 170 mg
Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 370 mg

Chelated iron

Na$_2$-EDTA $\cdot$ 2H$_2$O 37.3 mg
Ferrous sulfate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 27.8 mg

Microelements

Boric acid, $\text{H}_3\text{BO}_3$ 6.2 mg
Manganese sulfate, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 16.9 mg
Zinc sulfate, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 6.14 mg
Potassium iodine, KI 0.83 mg
Sodium molybdate, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.25 mg
Copper sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.025 mg
Cobaltous chloride, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 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 (Y₃) medium (1976).

**Macroelements**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium nitrate, KNO₃</td>
<td>2,020</td>
</tr>
<tr>
<td>Potassium chloride, KCl</td>
<td>1,492</td>
</tr>
<tr>
<td>Ammonium chloride, NH₄Cl</td>
<td>535</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate, NaH₂PO₄ · 2H₂O</td>
<td>312</td>
</tr>
<tr>
<td>Calcium chloride, CaCl₂ · 2H₂O</td>
<td>294</td>
</tr>
<tr>
<td>Magnesium sulfate, MgSO₄ · 7H₂O</td>
<td>247</td>
</tr>
</tbody>
</table>

**Chelated iron**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂-EDTA · 2H₂O</td>
<td>37.3</td>
</tr>
<tr>
<td>Ferrous sulfate, FeSO₄ · 7H₂O</td>
<td>13.9</td>
</tr>
</tbody>
</table>

**Microelements**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese sulfate, MnSO₄ · 4H₂O</td>
<td>11.2</td>
</tr>
<tr>
<td>Potassium iodine, KI</td>
<td>8.3</td>
</tr>
<tr>
<td>Zinc sulfate, ZnSO₄ · 7H₂O</td>
<td>7.2</td>
</tr>
<tr>
<td>Boric acid, H₃BO₃</td>
<td>3.1</td>
</tr>
<tr>
<td>Cobaltous chloride, CoCl₂ · 6H₂O</td>
<td>0.24</td>
</tr>
<tr>
<td>Sodium molybdate, Na₂MoO₄ · 2H₂O</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Appendix 2 (Continued)

Copper sulfate, CuSO₄ \cdot 5H₂O 0.16 mg
Nickle chloride, NiCl₂ \cdot 6H₂O 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, $\text{KH}_2\text{PO}_4$ 27.2 mg

Potassium nitrate, $\text{KNO}_3$ 101.0 mg

Calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1,480.0 mg

Manganese sulfate, $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$ 246.0 mg

Potassium iodine, $\text{KI}$ 0.16 mg

Copper sulfate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.025 mg

2-N-morpholinoethanesulphonic acid, MES 6,000.0 mg
Appendix 4


1. Prepare a stock solution of FDA (1-5 mg/ml acetone) and store at $0^\circ$ 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.

\[
\text{\% protoplast viability} = \frac{\text{no. of fluorescing protoplasts}}{\text{total no. of protoplasts}} \times 100
\]
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. Resediment 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 (Pfoser 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-MgCl₂ 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 H₂O
- 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 H₂O
- 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-MgCl₂ buffer stock solution

0.2 M Tris 4.84 g
4 mM MgCl₂ . 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
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.](image-url)
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.

<table>
<thead>
<tr>
<th>Reference plants</th>
<th>DNA content (pg 2C⁻¹) (Mean±SE)⁴</th>
<th>Reference plants (external reference)⁵</th>
<th>(Base pairs)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reference</td>
<td>Elaeis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plants</td>
<td>(external reference)</td>
</tr>
<tr>
<td><em>G. max</em> cv. Polanka</td>
<td>2.5²</td>
<td>3.77±0.09³</td>
<td>1.82×10⁹</td>
</tr>
<tr>
<td><em>L. esculentum</em> cv. Stupicke</td>
<td>1.96¹</td>
<td>4.25±0.09²</td>
<td>2.05×10⁹</td>
</tr>
<tr>
<td><em>Z. mays</em> line CE-777</td>
<td>5.72¹</td>
<td>4.72±0.23⁴</td>
<td>2.28×10⁹</td>
</tr>
</tbody>
</table>

¹Dolezel *et al.* (1992); ²Afza *et al.* (1993)  
³Base pairs per haploid genome (Bp 1C⁻¹ nuclei) calculated based on the equivalent of 1 pg DNA=965 Mega bp.  
⁴Values 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.

<table>
<thead>
<tr>
<th><em>Elaeis</em> cultivars</th>
<th>DNA content (pg $2C^{-1}$) (Mean±SE)*</th>
<th><em>Elaeis</em> (Base pairs)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. max</em> cv. Polanka</td>
<td>2.5$^1$</td>
<td>$1.21 \times 10^9$</td>
</tr>
<tr>
<td><em>Dura</em> (D109)</td>
<td>3.46±0.02$^b$</td>
<td>$1.67 \times 10^9$</td>
</tr>
<tr>
<td><em>Pisifera</em> (P168)</td>
<td>3.24±0.01$^c$</td>
<td>$1.56 \times 10^9$</td>
</tr>
<tr>
<td><em>Tenera</em> (T38)</td>
<td>3.76±0.02$^a$</td>
<td>$1.81 \times 10^9$</td>
</tr>
</tbody>
</table>

$^1$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

