OIL PALM LIQUID CULTURE - MPOB PROTOCOL

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As an alternative to culture on solid medium, the use of liquid culture for oil palm propagation has been attempted in order to explore its potential for automation and scaling-up propagule production as well as for improving growth and culture uniformity (de Touchet *et al.*, 1991; Teixeira *et al.*, 1995; Wong *et al.*,1999). However, a detailed protocol of the method has not been published to date. Therefore, the purpose of this technology transfer is the provision of the established MPOB protocol to interested parties.

OIL PALM LIQUID CULTURE PROTOCOL

Materials

1. Selection of suitable callus.

   Friable embryogenic callus is the best starting inoculum (*Figure 1*).

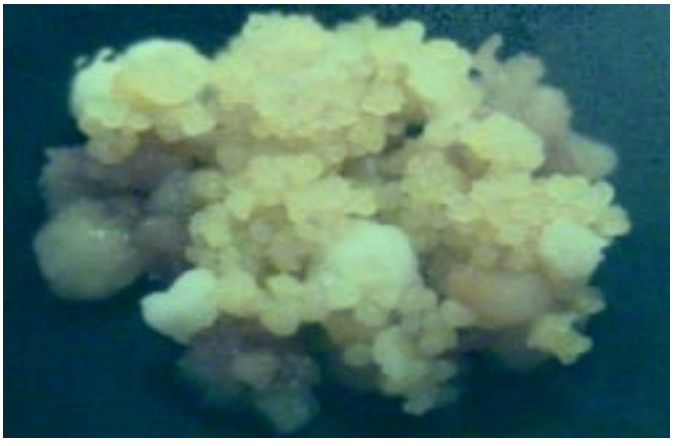

Figure 1. Friable embryogenic callus.

2 Media preparation.

Medium 1:

Liquid medium - for culture establishment and maintenance.

Basal medium: MS (Murashige and Skoog, 1962) macro, MS micro, Vitamins, 0.1 g litre^{-1} myo-inositol, 0.1 g litre^{-1} L-glutamine, 3% sucrose (castor sugar brand MSM).

Hormone: 1 mg litre^{-1} 2,4-D or 1 mg litre^{-1} 2,4-D + 0.1 mg litre^{-1} NAA.

Volume: 20 ml in 100 ml flask.

Medium 2:

Liquid induction medium - for maturation induction of culture aggregates.

Basal medium: MS macro, MS micro, vitamins, 0.1 g litre^{-1} myo-inositol, 0.1 g litre^{-1} L-glutamine, 3% sucrose (castor sugar brand MSM).

No hormone.

3. Preparation of apparatus for sterile work.

   Apparatus required are:

   - polypropylene sheets (10 x 10.5 cm);
   - beakers;
   - strainers (sieves) 2 mm, 1 mm, 500 µm, 106 µm mesh sizes;
   - forceps/scalpels/spatulas/scoops; and
   - all apparatus need to be autoclaved.

Methodology

1. Inoculation.

   a) Transfer 0.03-0.09 g of friable embryogenic callus into 20 ml of MS media.

   b) Replace cotton plugs with polypropylene sheets

   c) Transfer flasks to shakers (*Figure 2*).

   Physical conditions: dark room, temperature 27°C ± 1°C. Speed: 90 -100 rpm.

Figure 2. Flasks on shakers.

2. Subculturing at monthly intervals.

   Duration for establishment of liquid cultures may vary between clones (usually more than three subcultures) (*Figure 3*).

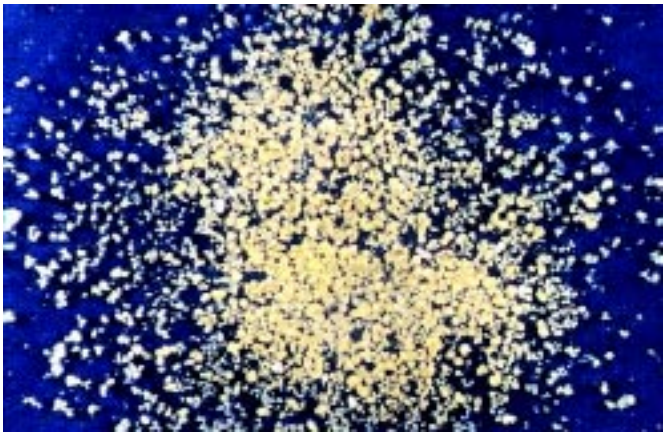

Figure 3. Established cell aggregates.

- Select cultures (various sizes);
- Streak to check contamination;
- Sieve according to sizes, *e.g.* 2 mm,1 mm, 500 µm; and
- Transfer 0.3-1.0 g of cell aggregates to 20 ml MS medium.

3. Maturation.

- Transfer to basal liquid MS medium to induce maturation before regeneration (one month).

4. Regeneration.

- Transfer cultures to basal MS solid medium (petri dishes, baby jars or flasks).

5. Multiplication of embryoids/shooting/rooting and establishment in soil will follow standard protocol as reported for solid culture system (Zamzuri *et al*., 1999).

CONCLUSION

This protocol has been established and used routinely at MPOB. However, the performance of the cultures is genotype dependent and the duration for establishment of liquid cultures varies from clone to clone.

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