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DEVELOPMENT OF THE MPOB FAST TRANSFER TECHNIQUE (MoFaTT) SYSTEM FOR MAINTENANCE AND MATURATION OF OIL PALM CULTURE AGGREGATES

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ABSTRACT

The MPOB Fast Transfer Technique (MoFaTT) in Liquid Culture System was developed as a rapid and convenient method for liquid media replenishment during maintenance and maturation of cultures. The system was developed with the aim to further improve the efficiency of the liquid culture system. Oil palm cultures can be maintained for three to four months, and replenishment of medium can be done on the shaker at any desired time. The usefulness of the MoFaTT is not just the rate of multiplication or proliferation of cultures but also the efficiency of the system compared to the conventional maintenance of cultures in individual shake flasks. Some of the benefits offered are a reduction in medium replenishment steps and the time consumed, on-site medium replenishment and improved practicality of the system.

Keywords: oil palm suspension cultures, MPOB Fast Transfer Technique (MoFaTT) in Liquid Culture System, replenishment, proliferation, multiplication.

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INTRODUCTION

The production of oil palm suspension cultures using the individual shake flask system have been established (De Touchet *et al*., 1991; Teixera *et al*., 1995; Wong *et al*., 1999; Tarmizi *et al*., 1999; Tarmizi, 2002). The protocols were developed to produce a reliable supply of regenerable plant tissues. The liquid culture system has also been developed to address the problem of inefficiency in micropropagation. However, the individual shake flask system which has been used routinely is inefficient for medium replenishment and culture transfer. This is because cultures which are to be subcultured have to be moved out from the culture room and transfer work has to be done in a sterile laminar flow cabinet. In order to overcome this problem, MPOB Fast Transfer Technique (MoFaTT) in Liquid Culture System was developed to enable a rapid and convenient way to replenish liquid media during maintenance and maturation of cultures. This further improves the efficiency of the liquid culture system.

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MATERIALS AND METHODS

Development of MoFaTT in Liquid Culture System

Materials. The system consists of the following items:

- 100 ml or 250 ml conical flasks (wide mouth) with two side arms;
- silicon tubing 6 x 8 mm;
- VENT devices 37 mm;
- ratchet clamp; and
- Y type connector.

***The set-up for MoFaTT requires the following*:**

- pre-sterilized flask with media for culture maintenance;
- an empty pre-sterilized flask for discarding spent medium;
- other pre-sterilized flasks with fresh maintenance, maturation or other specific media;
- clamps to attach to the tubing, to be released during medium replenishment; and

- filter devices attached to each flask to reduce the pressure build-up within the flask.

Plant Materials

The cultures used for testing the MoFaTT system were embryogenic suspensions with <2 mm aggregates from eight clones derived from elite palms. They were E34 (0189/2844), E80 (0.189/195), E90 (0.189/1173), E 104 (0.194/455), E110 (0.192/2202), E127(0.195/1085), E139 (0.366/123) and E141 (HDP56/385).

Media

The liquid MS (Murashige and Skoog, 1962) medium with 1 mg litre^{-1} nicotinic acid, 0.1 g litre^{-1} myo-inositol, 0.1 g litre^{-1} L-glutamine and 3% sucrose (castor sugar brand MSM) was used (Rohani *et al*., 2003). The medium was supplemented with 1 mg litre^{-1} dichlorophenoxyacetic acid (2,4-D) and 0.1 mg litre^{-1} 1-naphthaleneacetic acid (NAA). This hormonal combination was found effective in oil palm liquid culture system (Tarmizi, 2002). The pH of the medium was adjusted to 5.7 before autoclaving at 121°C for 25 min.

Inoculation of Culture and Maintenance in MoFaTT

Approximately 0.5 g of embryogenic suspension cultures were inoculated in 20 ml MS medium in MoFaTT. For comparison, suspensions from the same cultures were transferred to individual 100 ml flasks with an inoculation of 0.5 g per 20 ml of the same medium and both systems were incubated in darkness at 100 rpm on an orbital shaker. This shake flask system is used routinely for the proliferation of liquid cultures (Tarmizi, 2002).

RESULTS AND DISCUSSION

Development of the MoFaTT in Liquid Culture System

Modified flasks with side arms are interconnected with silicon tubings and placed on shakers. The arrangement of the system depends on the usage and nature of the experiments. One possible arrangement is shown in *Figure 1*. One flask is for maintenance of cultures (i) and the other two for the fresh maintenance (ii) and maturation (iii) media. One empty flask (iv) is also connected for discarding spent culture medium. Clips are attached to the tubing and released during replenishment. Filter devices are also attached to each flask to reduce the pressure build-up within the flasks. Replenishment is done by lifting the flask with appropriate medium about 15 cm higher than the base of the receiving flask with inclination of about 30° to 40° on the shaker (without having to take the flask to a laminar flow cabinet) at monthly intervals or a desired time of up to four months. The minimum number of flasks required is two flasks at any one time and their size could vary from 100 ml to 250 ml or larger.

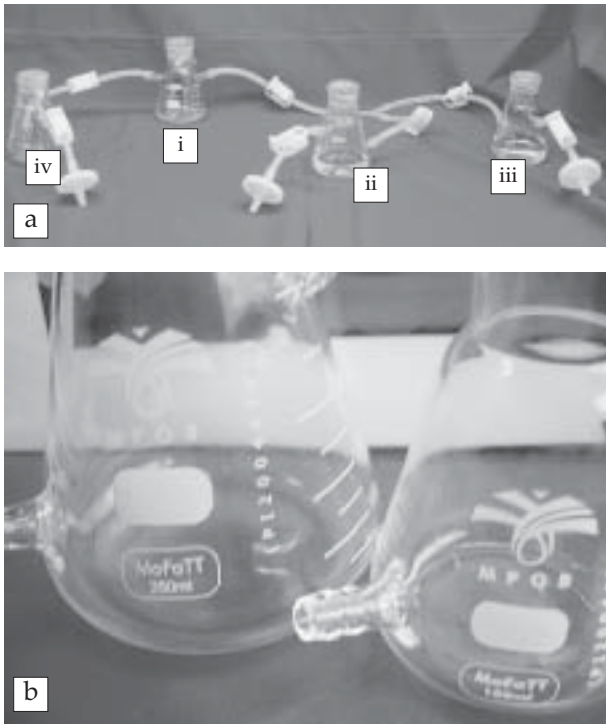

Figure 1. MPOB Fast Transfer Technique (MoFaTT). (a) One of the possible arrangements in MoFaTT and (b) individual flask with side arms.

The MoFaTT in Liquid Culture System was developed as a rapid and convenient method for liquid media replenishment especially during maintenance and maturation of cultures (*Figure 2*).

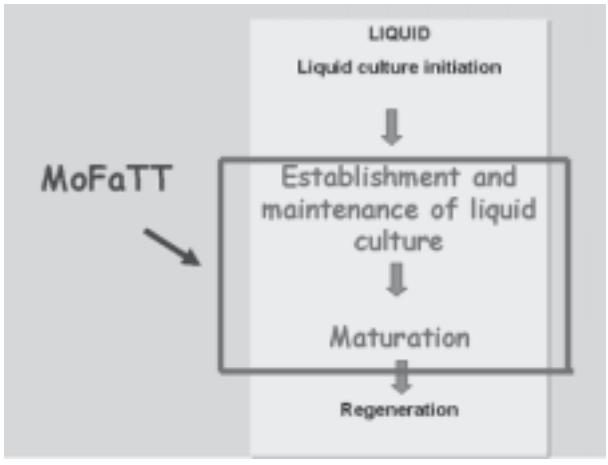

Figure 2. The application of the MoFaTT in liquid culture system.

Maintenance of Cultures in the MoFaTT System

Observation revealed that oil palm cultures could be multiplied in the MoFaTT system. The cultures could be maintained for three to four months and the replenishment of medium could be done on the shaker at any desired time; *e.g*. monthly. The fresh weight increment was better or comparable with normal maintenance in the individual shake flask system (*Table 1* and *Figure 3*). Cultures regenerated normally when transferred to solid media (*Figure 4*).

TABLE 1. INCREASES IN FRESH WEIGHT (X-fold) OF VARIOUS CLONES AFTER FOUR TIMES MEDIUM REPLENISHMENT IN THE MoFaTT AND SHAKE FLASK SYSTEM

Clone	MoFaTT	Individual shake flask
E 34	10 –fold (3.806 g)	7-fold (3.732 g)
E 80	8 –fold (9.023 g)	7 –fold (4.451 g)
E 90	3 –fold (1.690 g)	2 –fold (1.339 g)
E 104	4 –fold (4.077 g)	3 –fold (1.082 g)
E 110	10 –fold (5.977 g)	4 –fold (2.394 g)
E 127	3 –fold (1.830 g)	2 –fold (1.183 g)
E 139	7 –fold (3.313 g)	4 –fold (2.307 g)
E141	6-fold (6.886 g)	5-fold (3.157 g)
Mean	6.37-fold	4.25-fold

The Benefits of the MoFaTT System

The usefulness of MoFaTT is not solely based on the rate of multiplication or proliferation of cultures but on the convenience of the system compared to the conventional maintenance of cultures in individual shake flasks. The benefits offered were as follows:

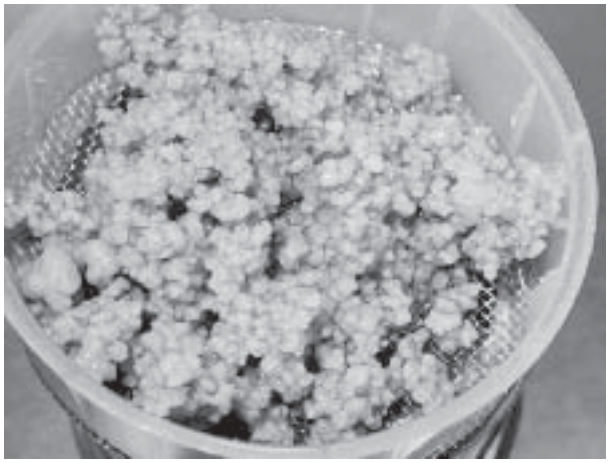

Figure 3. Some of culture aggregates collected after four months maintenance in MoFaTT system (1.1X).

Reduction in medium replenishment steps and time consumed. There are normally at least 10 steps with the conventional method of medium replenishment. It starts off with the opening of the polyethylene sheet (flask cover) of the flask with cultures which are to be transferred, flame sealing of the flask mouth part, discarding old medium, re-sealing of the mouthpart, repeating the same procedure for the flask with fresh media, replenishment, taking a new sterile polyethylene sheet, a final flame seal of the mouth part and finally covering it with the polyethylene sheet (*Figure 5*). However, the whole process is reduced to only two steps in MoFaTT system by just lifting the specific flask for discarding and medium replenishment. This is basically based on a gravity-feed principle, for movement of liquid. The most significant difference between the conventional medium replenishment method and MoFaTT system is the time consumed for medium

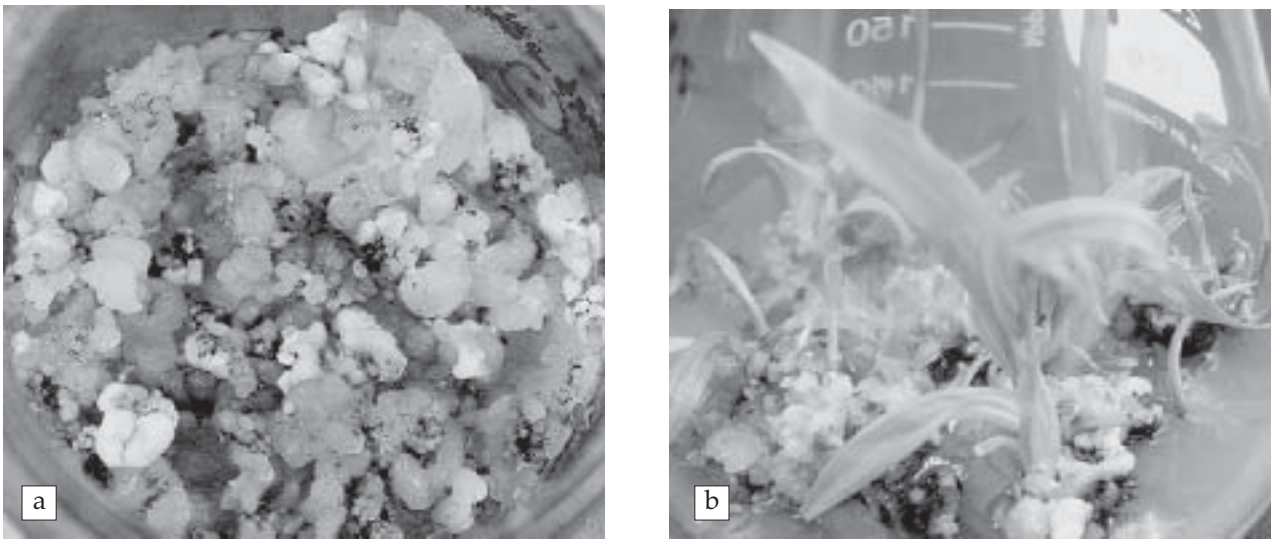

Figure 4. Cell aggregates of clone E34 from MoFaTT starting to regenerate (a) and proliferate shoots (b).

replenishment. It would take about 11 min to transfer medium under the conventional method, whereas it would only take about a minute or so for medium replenishment by MoFaTT system. The laboratory operator could only replenish about five flasks of cultures hourly, whereas by MoFaTT system one could transfer more than 30 flasks within the same period of time.

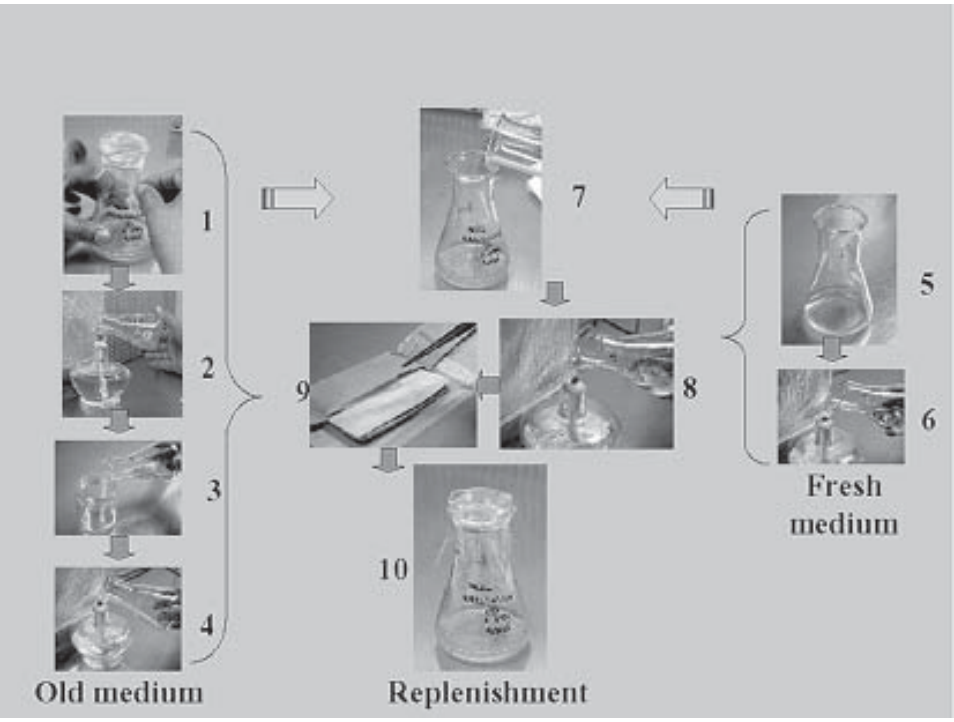

Figure 5. Conventional method of media replenishment during maintenance and maturation of cultures.

On-site medium replenishment. Under the conventional method for medium replenishment, flasks with cultures must be brought out from the culture room and transferred in a sterile laminar flow cabinet (LFC). The time taken for this activity has been omitted with MoFaTT system. In MoFaTT, the medium replenishment could be done on the shaker inside the culture room itself. Since, there is no movement of cultures from culture room to laminar flow cabinet (*Figure 6*) and opening of cultures, the problem of contamination of cultures could be avoided and thus, the risk of losing important cultures is much reduced. Contamination in *in vitro* plant cultures is critical and was discussed in detail regarding sources and solutions by Herman (2004).

Practicality of the system. The MoFaTT system is not only meant for oil palm cultures. It could be applied to any fluid culture system, whether plant, animal or microbial. Since there is no movement of cultures, the medium replenishment can be done easily and quickly at any desired time. Quick replenishment at daily, weekly, monthly or even hourly intervals can be done to look at effects on the culture development. This approach is almost impossible and impractical when using the conventional method. Some cultures sometimes need fresh medium to be added urgently to avoid further browning and this problem can be easily overcome using the MoFaTT system. All these advantages confirm the practicality of MoFaTT.

Figure 6. Medium replenishment on the shaker.

CONCLUSION

Various technologies have been developed in efforts to further improve the oil palm tissue culture system. The liquid culture system has been developed to address the problem of inefficiency in micro-propagation. The growth of plant cells is more rapid in suspension than in callus cultures and also more readily controlled because the culture medium can be easily amended or changed. For these reasons, suspension culture is expected to provide a means of very rapid plant multiplication (George, 1993). The basic protocol using the shake flasks system has been established and the bioreactor technique has also been developed in order to further improve the liquid culture system (Tarmizi *et al*., 2003). MoFaTT in Liquid Culture System was developed as a rapid and convenient method for liquid media replenishment during maintenance and maturation of cultures in the shake flask system. This further improves the efficiency of the liquid culture system. The system can also be applied to any fluid system. Various experimental approaches could be designed using the MoFaTT system such as intermittent medium replenishment, on-site application of various exogenous treatments to cultures, *etc*.

This technology can be exploited for further enhancement of a semi- or fully-automated processes for oil palm clonal production.

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