Somatic Embryogenesis of Oil Palm (*Elaeis guineensis* Jacq.) for Synthetic Seed Production

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ABSTRACT---- ***Somatic embryogenesis of oil palm in liquid medium has been developed since 1999 by Touchet et al. However, synthetic seed production of oil palm has not been performed. Synthetic seed is useful for long time storage and low cost delivery to far away plantation. In the present study, methods from somatic embryogenesis up to synthetic seed production were performed. Friable embryogenic callus was induced, embr yogenic cell suspension was initiated, development and maturation of somatic embryo were conducted. The maturation of somatic embryos were performed in two step. First step was inducing the accumulation of storage protein by arginine and glutamine. Second step was inducing dessication tolerance by Abscisic acid. After the somatic embryos maturing, they were dessicated for 2 hours on sterilized filter paper. Then, dessicated somatic embryo were mixed in 3% alginate solution and dropped one by one to 100 mM*** $CaCl_2$ ***solution to form beads of synthetic seeds. The synthetic seeds were then germinated on germination medium containing gibberelic acid. The synthetic seeds germinated after one month on germination media and on sterilized soil.***

Keywords--- Somatic embryogenesis, oil palm, synthetic seed

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1. INTRODUCTION

Oil palm is very useful for food and medicinal ingredient, such as margarine, palm oil, omega 6. Therefore, it is very important to propagate the oil palm biotechnologically, such as by micropropagation method.

Micropropagation via somatic embryogenesis can be used for clonal propagation and in vitro conservation. Regenerated plants were true-to-type and resulting in uniform plants. (Jayanthi and Mandal, 2001; Tokuhara and Mii, 2001). Somatic embryogenesis is advantageous for mass propagation, genetic improvement programs and production on synthetic seeds (Hartmann *et al*., 1997). Somatic embryogenesis is an amazing process because the bipolar structure processing shoot and root resembling zygotic embryo, is produced from somatic cells.(Mariani *et al*., 1998).

Most of the somatic embryogenesis technology was developed on semisolid medium. There are few reports on somatic embryo development and maturation from liquid medium.(Gupta *et al*., 2000). The technology of liquid culture save almost all the laboratorium operational cost, i.e. labour, time, place, and chemicals. Quality of the products is also improved in liquid medium compared to semi-solid medium (Gupta *et al.*, 2003). In liquid medium, somatic embryo coud be produced as much as 1000 embryo/ liter medium (1 embryo/ ml medium). (Osmotek Ltd, 2002).

Seeds represent the ultimate convenience for crop production due to their ease of use and low cost when compared with any other form of propagule. Seeds possess durable, protective coatings and are often dehydrated and quisescent, allowing simplified handling and storage. Synthetic seeds are functionally defined as somatic embryos engineered to be of use in commercial plant production. (Gray and Purohit, 1991).

High volume propagation potential of somatic embryogenesis combined with formation of synthetic seed for low-cost delivery would open new field for clonal propagation.(Redenbaugh *et al.*, 1987). Candidate crops for synthetic seed production can be classified into two categories : 1) those that have a strong technological basis, such that high quality somatic embryos can currently be produced, and 2) those with a strong commercial basis (Redenbaugh *et al.*, 1987).

Oil palm fulfill the two categories above, because high qualities of oil palm somatic embryogenesis has been performed successfully (de Touchet et al., 1991; Teixeira *et al.*, 1993, 1995, Aberlenc-Bertossi, 1999) and have strong commercial basis.

2. MATERIAL AND METHOD

2.1. Material

Material used in this study was embryogenic calli 638 clone from Marihat, North Sumatra, Indonesia. The composition of medium is listed in table 1.

Tabel 1. Composition of medium for oil palm somatic embryogenesis

No	Step of somatic embryogenesis	Medium	Hormone or supplement	cubation period
1	Initiation	SIM	2,4-D 226 µM; BAP 4,44 µM	4 weeks
2	Development I	EDM I	-	4 weeks
3	Development II	EDM II	BA 5 µM	1 week
4	Maturation I	MM I	glutamine 20 mM; arginine 5 mM	3 weeks
5	Maturation II	MM II	ABA 25 µM	2 weeks

Keterangan: The basal medium wan Touchet medium. SIM = Suspension initiation medium; EDM I = Embryo development medium I; EDM II = Embryo development medium II; MM I = Maturation medium I; MM II = Maturation medium II.

2.2. Initiation of embryogenic cell suspension culture

Suspension cultures were initiated by inoculating 500 mg friable embryogenic callus into 20 ml suspension initiation medium (SIM) in 100 ml flask. The flasks were placed on a gyratory shaker at 80 rpm. The culture condition was 27 ± 1^{o}C, light intensity 20 µmoles $m^{-2}sec^{-1}$ and 12 hr photoperiod.

2.3. Development of somatic embryo

After 4 weeks of culture in SIM, the somatic embryos were subcultured into embryo development medium I (EDM I) for 4 weeks. Subsequently, the globular somatic embryo were sieved using 2 mm nylon mesh and plated on embryo development medium II (EDM II) for 1 week.

2.4. Maturation of somatic embryo

Developed somatic embryo were then subcultured on maturation medium I (MM I) containing arginine and glutamine for 3 weeks. For desiccation tolerance, the somatic embryo were subcultured on maturation medium II (MM II) containing abscisic acid for 2 weeks.

2.5. Synthetic seed

Matured and dessication tolerance somatic embryo were dessicated for 2 hours. Thereafter the somatic embryos were mixed in sodium alginate solution. Subsequently the somatic embryos were pipetted and dropped one by one into calcium chloride solution. The beads containing somatic embryos were formed and they were kept in the calcium chloride solution for 24 hours in 25^{o} C. The synthetic seeds were then cultured on germination medium.

3. RESULT AND DISCUSSION

3.1. Embryogenic callus

Figure 1 shows friable embryogenic callus of oil palm clone 365. Cells in nodules at the surface of embryogenic callus was undegoing high proliferation cell and formed meristematic cell agregate, which have the potency became embryogenic cells in the suitable medium. (Filho and Hattori, 1997)

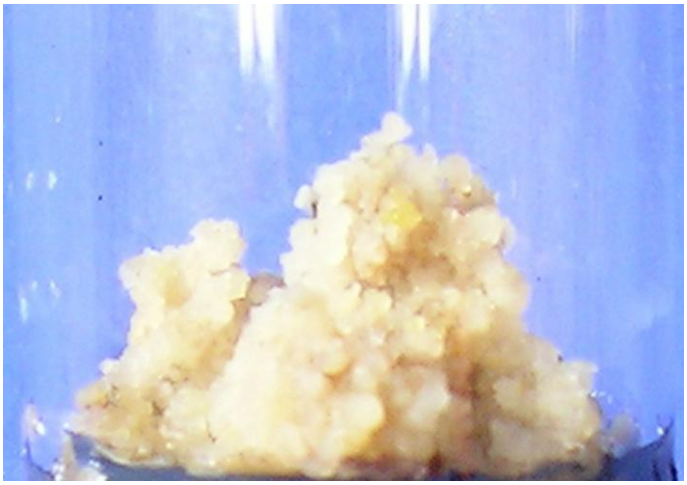

Figure 1. Embryogenic callus of oil palm clone 638

3.2. Initiation of embryogenic cell suspension

Inoculum to initiate embryogenic cell suspension culture was the friable embryogenic callus. The result showed that single embryogenic cell was obtained in both medium. The single embryogenic cell is round and small, has dense cytoplasm, large nucleus, little vacuole and thin cell wall (Fig. 2). This single embryogenic cell divided asymmetrically within 5 days of culture as shown in Fig. 3. The two smaller cell will become embryo and the bigger cell will become suspensor. This asymmetric division is an important character of somatic embryogenesis. Fig. 4 shows globular somatic embryo with suspensor within 2 weeks of culture. The existence of the suspensor gave the evidence that the embryo derived from a single cell. Mariani et al. (2000) reported that at an early stage, suspensors were observed on the elongated somatic embryo, because the somatic embryo was unicellular origin. Unicellular origin means that the somatic embryo was derived from a single cell. Suspensor in somatic embryo was also found in *Phaseolus vulgaris* (Puspitawati 1997), *Vigna radiata* (Puspitawati, 1997; Fitriani, 2002) *Allium sativum* (Nurwendah, 2002) dan *Lithospermum erythrorhizon* (Ramayanti, 2003).

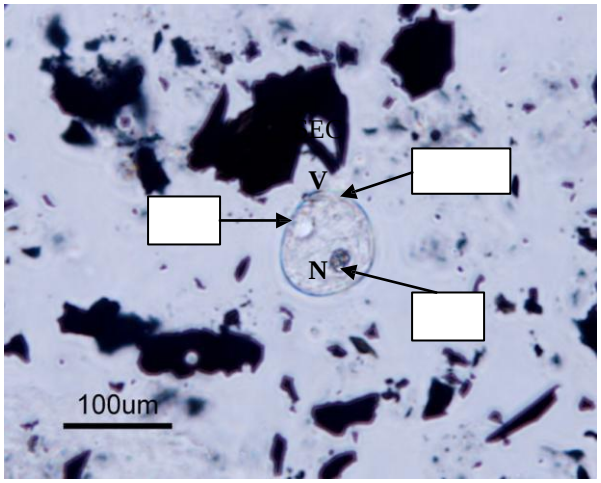

Fig. 2 Single embryogenic cell (SEC) of oil palm *Elaeis guineensis*, Jacq. Clone 635. V=Vacuole, N=Nucleus

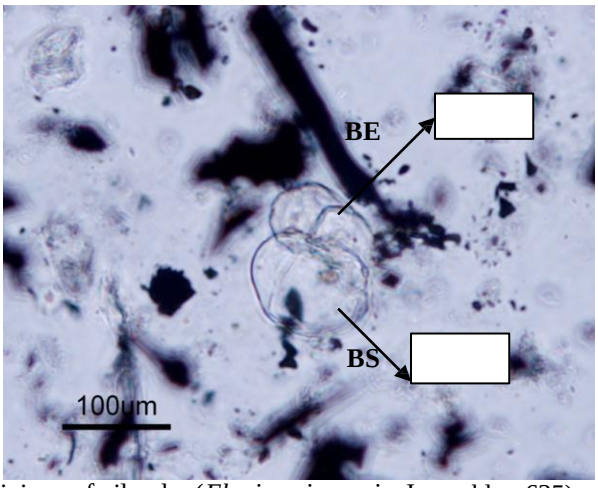

Figure 3. Asymmetric divison of oil palm (*Elaeis guineensis*, Jacq.. klon 635) cell (BE = Becoming Embryo ; BS = Becoming Suspensor)

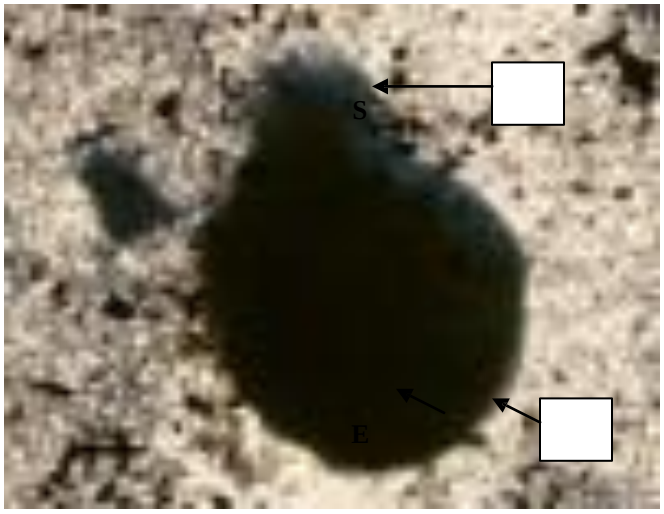

Figure 4. Globular somatic embryo with suspensor from Embrio oil palm (*Elaeis guineensis*, Jacq.Clone 635) (S = Suspensor ; E = Embryo)

According to our result, the somatic embryo of oil palm clone 635 underwent direct somatic embryogenesis derived from single cell. This is coinciding with Noerhadi (1988) that direct somatic embryogenesis was the formation of embryos from single cell. This is advantageous because it can reduce somaclonal variation. In addition, this advantage will be important in applying the somatic embryo in plant genetic transformation (Mariani *et al*., 2002).

3.3. Synchronization of embryo

Synchronization of embryos were conducted by selecting the globular somatic embryos in suspension initiation medium (SIM) 4 weeks of culture by using 2 mm nylon mesh sieve. Sieving process was needed to obtain efficient and synchrone embryo size. (Kreuger, 1996; Tahardi, 1997 ; Bertossi *et al*, 1999). The key point in establishing such systems is the initial materials used, which should be homogenous cells having high competency. (Komamine, 2003). A high-frequency, synchronous embryogenic systems in liquid culture is needed to take full advantage of somatic embryogenesis as it is essential for automation and for investigating physiological, biochemical and molecular aspects of a process for which there is still limited information concerning woody tree

species (Tonon *et al*, 2001.) Synchronization of embryo in high frequency could be used as a basic for mass production of the embryo (Osuga dan Komamine ,1994).

3.4. Development of somatic embryo

Syncronized somatic embryo developed in Embryo development medium I (EDM I) without plant growth regulator. (Fig. 5). This is coincide with Hartmann (1997) that the somatic embryo developed on the medium without plant growth regulator. This embryo has epidermized and become one of the most important character in somatic embryo.

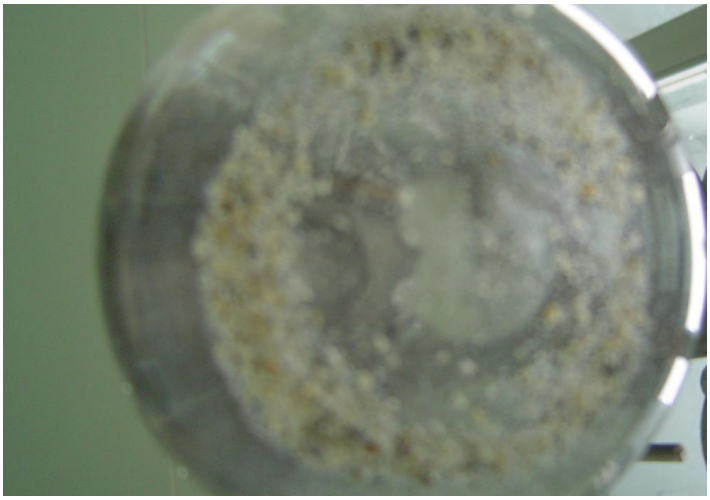

Figure 5. Developing embryo in Embryo Development Medium I (EDM I)

Globular embryo in EDM I subcultured onto Embryo Development Medium II (EDM II) containing BA is shown in Fig.6 Aberlenc-Bertossi et al. (1999) reported that the addition of BA during development was found to induce shoot apex differentiation and thus increased germination rates, by up to 70%.

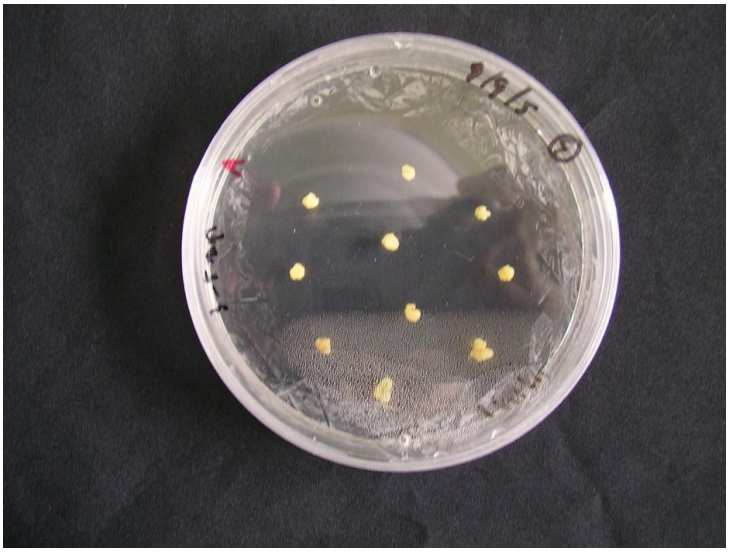

Figure 6. Epidermized embryo one week on EDM II

3.5. Somatic embryo maturation

Globular somatic embryo one week on EDM II (1-2 mm) was subcultured into Maturation Medium I (MM I) for maturation process. Maturing embryo became bigg er (6-7 mm) as shown in Figure 7. MM

containing arginin and glutamin amino acid that have function to induce storage material such as protein, lipid and starch. This storage material have function as energy source and will be metabolized during germination of the embryo. In addition, the embryo containing high storage material will be vigour. McKersie (1995) reported that in *Medicago sativa* maturaton phase I is considered to be the rapid growing in phase during most storage reserve deposition occurs.

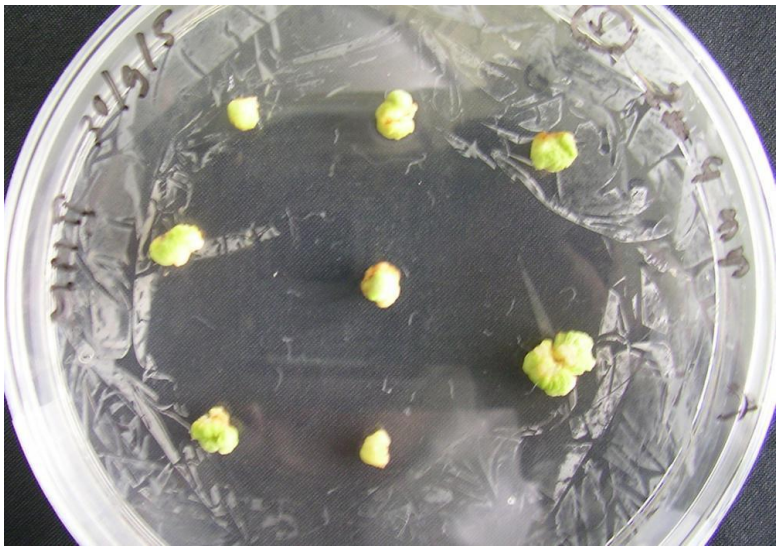

Figure 7. Maturing embryo 3 weeks on Maturation Medium I (MM I)

Maturing embryo on MM I was subcultured onto Maturation Medium II (MM II) containing abscisic acid for inducing desiccation tolerance as shown in Fig. 8. McKersie. (1995) reported that in *Medicago sativa* the induction of desiccation tolerance occurs in maturation phase II. Desiccation tolerance somatic embryo equal to the original seed and will enter dormancy process prior to germination process. Desication tolerance somatic embryo is a prerequisite for synthetic seed production.

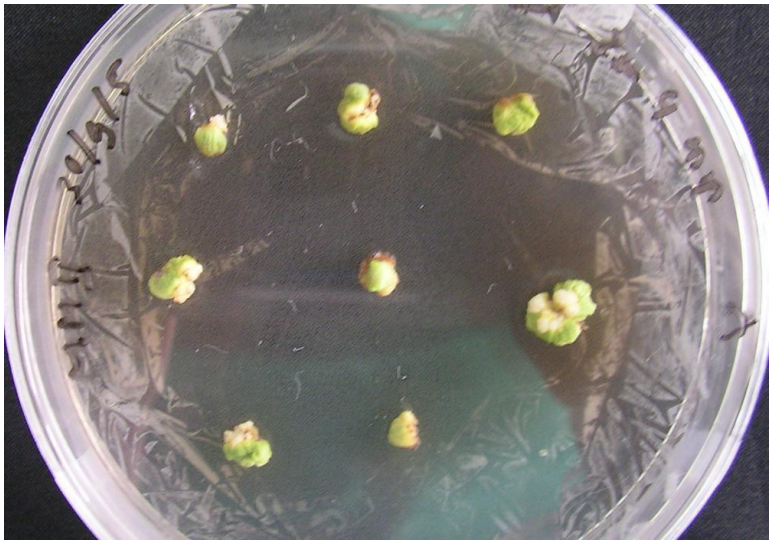

Figure. 8. Desiccation tolerance embryo 2 weeks on Maturation Medium II (MM II)

3.6. Synthetic seed

Synthetic seed has been made. Using 3% alginate and 1.5% maltose was suitable for encapsulation of embryo to form synthetic seed (Fig. 9). According to our knowledge, synthetic in oil palm in this study is firstly

reported. Synthetic seed in carrot (Timbert et al., 1995), Siberian ginseng (Choi and Jeong, 2002) alfalfa (Fujii et al., 1992) have reported as well.

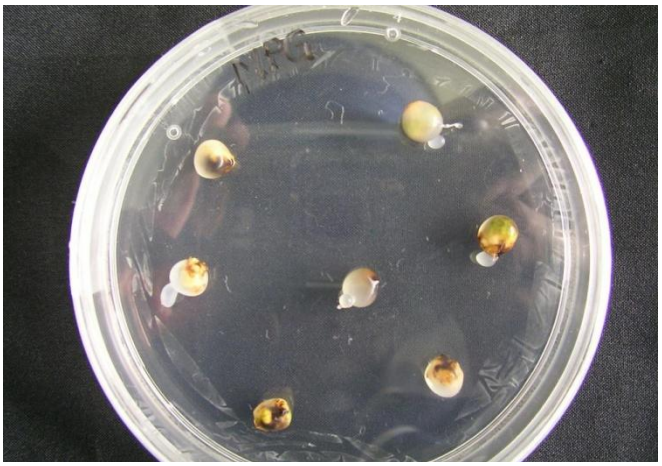

Figure 9. Synthetic seed of oil palm

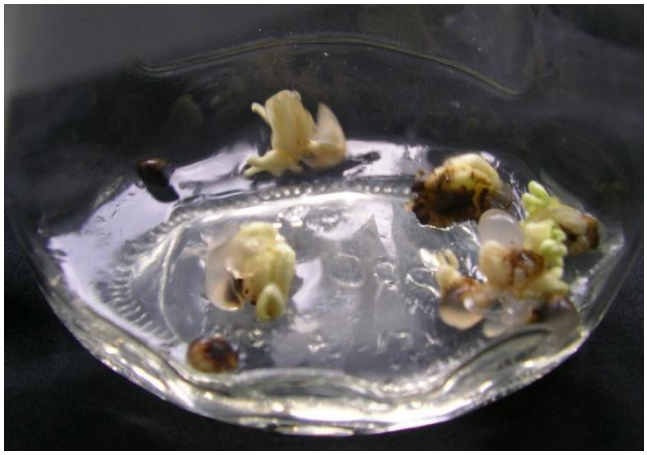

Figure. 10. Germinated synthetic seed of oil palm

Figure 11. Germinating synthetic seed of oil palm on sterilized soil

Fig. 12. Oil palm 638 clone in pre-nursery

The germinated synthetic seeds of oil palm were acclimatized (Fig. 12). Then, the oil palm plants were moved to plantation, flowering and set fruit.

In this study, the oil palm synthetic seed could germinated. It was due to the well physiological condition of oil palm during somatic embryogenesis.

4. CONCLUSION

The synthetic seeds of oil palm can be produced through several process, namely embryogenic callus induction, initiation of embryogenic cell suspension, synchronization of embryo, development of embryo, maturation I of embryo, maturation II of embryo, and finally encapsulation of embryo. The synthetic seed could germinated. The somatic embryo underwent direct somatic embryogenesis from single cell. Therefore, it is expected that somaclonal variation can be reduced.

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