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, embryogenic 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. 70% of synthetic seeds were germinated after one month on germination media.

Key words: Somatic embryogenesis, oil palm, synthetic seed

Introduction

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.

Material and method

1. Material

   Material used in this study was collected from Marihat, North Sumatra. Explant used was young leaf from oil palm clone 635.

2. Friable embryogenic callus induction

   Young leaves number–4,-5,-6,-7 and –8 were used as the explant. Sterilized leaves were cultured on embryogenic callus induction medium (ECIM) for 3 months and kept at 27^{o} Celcius in dark condition.

3. 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.

4. 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.

5. 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.

6. 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.

   Note: The medium of oil palm somatic embryogenesis is not explained in detail here because we are planning to make a patent of it.

Result and Discussion

1. Synchronization of embryo

After 3 months in Embryogenic Callus Induction Medium (ECIM), creamy, friable embryogenic callus were formed. Inoculum to initiate embryogenic cell suspension culture was the friable embryogenic callus. The result showed that single embryogenic cells and globular embryos were obtained in suspension initiation medium.

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).

2. Development of somatic embryo

Syncronized somatic embryo developed in Embryo development medium I (EDM I) without plant growth regulator. (Fig. 1). 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.

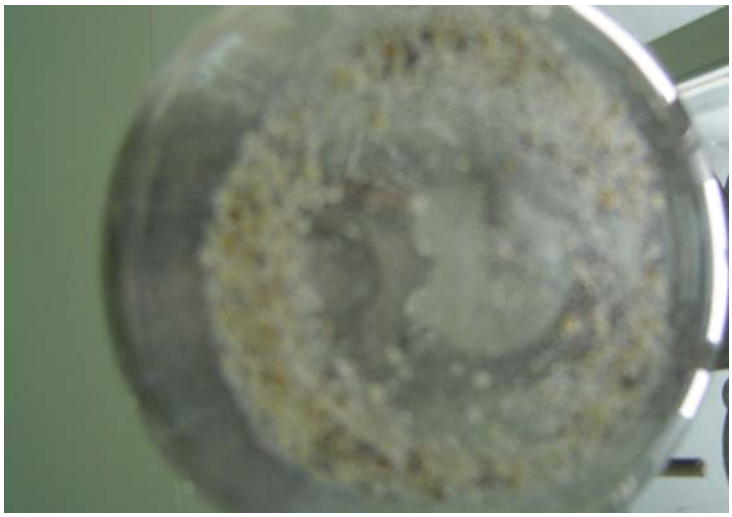

Figure 1. Developing embryo in Embryo Development Medium I (EDM I)

Globular embryo in EDM I subcultured onto Embryo Development Medium II (EDM II) containing BA is shown in Fig.2. 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 2. Epidermized embryo one week on EDM II

3. 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 bigger (6-7 mm) as shown in Figure 3. 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 3. 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. 4. McKersie. (1995) reported that in *Medicago sativa* the industion 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.

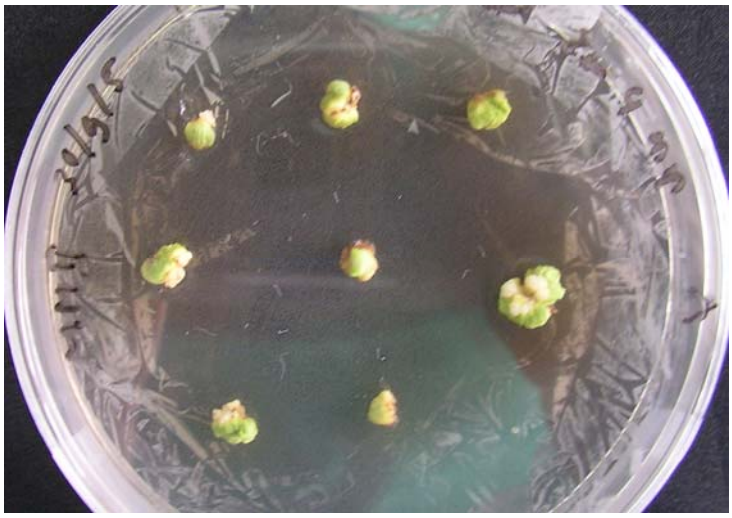

Fig. 4. Desiccation tolerance embryo 2 weeks on Maturation Medium II (MM II)

4. 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. 5). 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 5. Synthetic seed of oil palm

Fig. 6. Germinated synthetic seed of oil palm

In this study, 70% of the synthetic seed could germinated. Timbert et al. (1995) reported 30% to 35% germination of carrot synthetic seeds. Therefore, our germination percentage of oil palm synthetic seed was higher.

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 germination was 70%. The somatic embryo underwent direct somatic embryogenesis from single cell. Therefore, it is expected that somaclonal variation can be reduced.

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