SEM Study on Early Stages of Oil Palm (*Elaeis guineensis* Jacq.) Somatic Embryos

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ABSTRACT
Oil palm (*Elaeis guineensis* Jacq.) is a plant with highest productivity of oil among others oil-producing plants with total product per year is 5-6 ton/ha. Micropropagation of oil palm by somatic embryogenesis has several advantages: homogenous plants, higher production of fresh fruit bunches and larger amount of high quality seeds in a relatively shorter time. Oil palm 635 clone (15 years old) from Marihat Research Station, North Sumatra was used in this research. Embryogenic cell suspension was established by inoculating friable callus into suspension initiation medium (SIM) supplemented with 100 ppm 2,4-D and 1 ppm BAP. After four weeks of culture in SIM, the single embryogenic cells differentiated into proembryos and globular somatic embryos. Thereafter, the embryos were fixed and observed by Scanning Electron Microscopy (SEM) to reveal surface structure and morphology of the embryos. Initially, the proembryos cell has smooth surface, then deposition of fibrilar material occurred on the old cell wall surface, which become excessive during its development. The deposition of fibrillar material covered the cell wall surface and new cell wall was established. The rapid growth of proembryos caused degradation of cell wall and triggered the cell wall turnover. Proembryos stage was indicated by the appearance of extracellular matrix called supraembryonic structure (snt). Oil palm embryo underwent first tissue differentiation at globular stage, which was observed through a protoderm covering the cell walls. Deposition of cuticular layer component also occurred at this stage. This component similar to cuticular wax crystal found in *Arabidopsis*. The globular embryo of oil palm also had a suspensor structure with snt structure on it. This phenomena indicated that the suspensor growth was inhibited on a proembryo-like stage.

Key words : Oil palm, somatic embryo, fibrillar material, supraembryonic structure, cuticular wax crystal

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an oil–producing plant that play a role in international market and very important for industial in Indonesia. Production of palm oil per ha can achieve six ton per year (Sastrosayono, 2003). Both of crude palm oil and palm kernel oil can be used as food industrial material (fry oil and margarine), textile industry, cosmetics industry and as an alternative biodiesel.

Generally, oil palm are produced by generative propagation using seed. This kind of propagation resulted in fenotypic variation and induced variation in plant productivity. Vegetative propagation is a way that should be taken to obtain progenies, which fenotypically and genotypically similar to its parental. The benefit of somatic embryogenesis in propagating the oil palm has been proven to be succeed (Jones, 1983; Blake, 1983; Litz *et al.*., 1985 *in* Pierik, 1997) and could produce homogenous clone that has homogenous productivity as well.

Somatic embryogenesis is an amazing process because the bipolar structure possesing shoot and root resembling zygotic embryo, is produced from somatic cells. (Mariani *et al.*, 1998). Somatic embryogenesis can be performed in solid media as well as liquid media (suspension culture).

Nevertheless, the use of liquid or cell suspension culture system will reduce the time frame, labour input and consequently the cost for plantlet production (Tahardi, 1997).

Observations of somatic embryo development by stereo microscopy gave us only information concerning the change of their shape but those by SEM (scanning electron microscopy) observations led us more detail information concerning their surface changes during development (Mariani *et al*., 1998). Observation of the changes of the cell wal surface during the somatic embryogenesis in suitable culture conditions by SEM shall contribute to the elucidation of the mechanisms involved in somatic embryogenesis of certain species.

Mariani *et al.* (1998) used SEM to observe changes of surface structure in detail in direct somatic embryogenesis of *Oryza sativa* L. SEM also used by Sato *et al.* (2001) to observe callus surface of *Oryza sativa* L. treated by cell wall degrading enzymes. Taylor *et al.* (1996) observed ultrastructure of *Pennisetum glaucum* somatic embryo development by SEM for comparative study with the ultrastructure of zygotic embryo from the same species. However, to my knowledge, observation on surface ctructure and morphology of oil palm somatic embryo produced by direct somatic embryogenesis has not

been performed yet. Therefore, the objective of this study is to make description concerning surface structure and morphology of early stages of oil palm somatic embryo observed by SEM.

MATERIALS AND METHODS

1. Materials

The explants were young leaves, namely : -4,-5,-6,-7,-8 from the oil palm ortet.
The ortet was obtained from Marihat Research Station, Pematang Siantar, North Sumatra.

2. Medium

Medium used were embryogenic callus induction medium (ECIM), suspension initiation medium (SIM). Basal medium was Touchet medium (1991). The composition of the medium was shown in Table 1.

3. Methods

3.1. Embryogenic callus induction

Young leaf explants were cut with size 1.5 x 1.5 cm. Then, they were sterilized with 0.26% Sodium hypochlorite for 20 minutes. Thereafter, all explants were dipped in 2% glucose for 20 minutes and sowed on ECIM. The cultures were maintained in 25 °C in the dark. After 3 months, friable embryogenic calli were obtained.

3.2. Somatic embryo initiation

0.5 g friable embryogenic calli were inoculated into 20 ml suspension initiation medium (SIM). The cultures were shaken at 80 rpm, maintained in 25°C and 12 hr photoperiod for 4 weeks. Proembryo and globular somatic embryos were collected to be fixed for SEM observation.

3.3. Scanning Electron Microscopy (SEM)

Proembryos and globular somatic embryos were prefixed with 5% glutaraldehyde in cacodylate buffer pH 7.2 at 4^{o}C for 24 hr. Samples were rinsed in 0.1 M cacodylate buffer 3 times and distilled water once. Thereafter, the samples were successively gradually dehydrated in an alcohol series, immersed in isoamyl acetate for 5 min and dried at the critical point in 'Critical Point Drier' Denton Vacuum DCP-1 using CO_2 as the transient fluid. Finally, the samples were coated with gold by JEOL JEE sputter coater and observed by SEM Philips XL 20 (Erlangga, 2006).

Table 1. Composition medium of ECIM and SIM

Medium	Plant growth regulator		Activated charcoal	Gelling agent
	2,4-D (ppm)	BAP (ppm)		
ECIM	200	-	0,3%	Phytagel 0,25%
SIM	100	1	0,1%	-

RESULT AND DISCUSSION

1. Proembryo

Proembryo was the earliest stages in somatic embryogenesis, when protoderm has not been formed yet. The oil palm proembryos in this study were formed from single cells that were dispersed in suspension initiation medium (SIM). SIM containing 100 ppm 2,4-D and 1 ppm BAP was the optimum medium, which enable to initiate embryogenic cells and differentiated further to form proembryos and globularr somatic embryos (Wardjo, 2006).

Differentiation of proembryos and globular somatic embryos from single embryogenic cells were possible due to the existence of BAP (cytokinin) in the medium. Pierik (1997) stated that the addition of cytokinin could help the process of somatic embryogenesis.

Result of SEM observation on one cell of oil palm proembryo showed a smooth surface structure (Figure 1.a). Similar surface structure was also observed by Mariani *et* al (1998) on rice (*Oryza sativa* L.) proembryo and by Nurwendah (2002) on garlic (*Allium sativum* L.) proembryo. Figure 1 a. showed a phase where cell wall covered the proembryo cell intactly before the cell wall undergo breakdown and regeneration. The cell wall can undergo breakdown and regeneration due to the character of proembryo cell that continuously grow and divide leading to the globular stage formation.

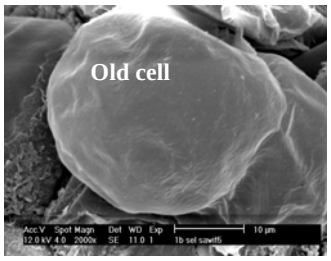
2. Regulation of cell wall during proembryo stage

2.1. Cell wall formation

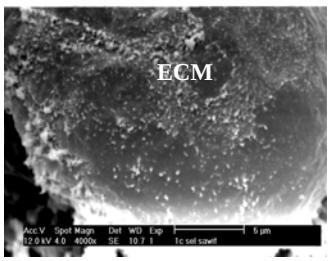
Process of embryo growth from proembryo leading to globular stage was characterized by the increasing of cells amount and cells volume. Morphologically, this process can be seen from cell wall formation and covering of old cell wall by new cell wall. Cell wall is an extracellular matrix (ECM) in the plant. It is quite complex but easily to observe morphologically so that cell wall observation help to understand ECM regulation in the plant.

Figure 1.b and 1.c showed the process of cellulose microfibril movement synthesized in the enzyme complex called rosette. This cellulose microfibril is included into ECM. Similar structure was shown by Nurwendah (2002) on the transition phase of garlic somatic embryo.

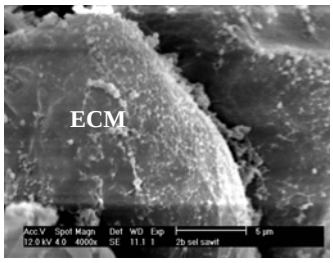
Figure 1.d showed that the rosette were not secreting the cellulose microfibril anymore. It was indicating that microfibril material for cell wall formation has enough. Fosket (1994) mentioned that eventhough the biochemical synthesis of rosette complex up to now has not yet known, the structure can be seen by electron microscopy.



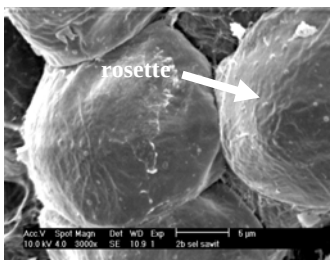
a. Before ECM secretion



b. Pre-secretion of ECM



c. Post-secretion of ECM



d. New cell wall has been formed

Figure 1. Stages of cell wall formation in proembryo cell of oil palm (*Elaeis guineensis* Jacq.) 635 clone. ECM = Extracellular matrix.

2.2 Supraembryonic network

Chapman *et al.* (2000) reported that there was extracellular matrix structure called 'supraembryonic network' (*snt*) on the surface of *Cichorium*, *Citrus*, dan *Asparagus* proembryos. This structure disappeared when protoderm was formed in the embryo surface. Proembryo stage in the oil palm showed similar structure connecting one cell with another cell in the proembryo. (Figure 2).

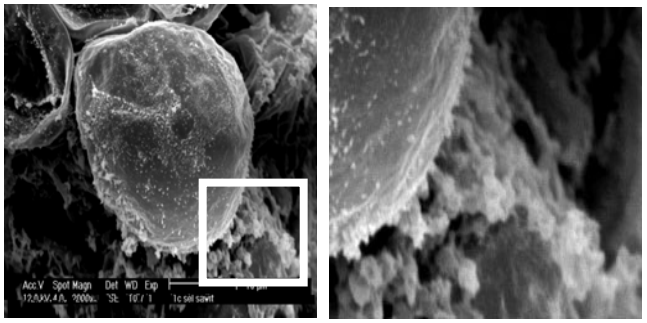


Figure 2. One proembryo cell with snt structure (left). Inset is the magnification of snt structure (right). snt=supraembryonic network.

By reffering to the observation of cell wall ultratsructure (Mariani *et al.* , 1999; Chapman *et al.*, 2000), it was known that extracellular matrix and snt is the same, i.e. fibrillar material. Chemical composition of the snt is not yet known, but according to Samaj *et al*. (1995) one of the material of *snt* in *Drosera rotundifolia* dan *Zea mays* was proteoglycan.

3. Globular somatic embryo

3.1 Embryo morphology

The difference between proembryo and globular embryo was in their size. The diameter of proembryo was < 200 µm whereas that of globular embryo was > 200 µm. Figure 3 showed the morphology of globular oil palm somatic embryo with the diameter more than 500 µm. The characteristic of globular embryo was the formation of epidermal tissue. Epidermis differentiated from the meristematic surface layer, i.e. protoderm. Protoderm derived from periclinal division in the transition stage from proembryo to globular stage (Figure 4). In this stage, protoderm started to produce wax, which was the typical character of the protoderm when it was differentiated into epidermis (Fosket, 1994).

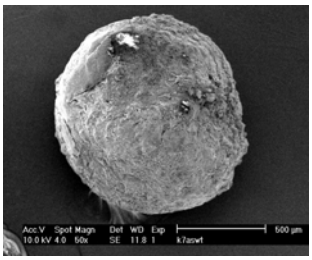


Figure 3. Globular somatic embryo of oil palm (*E. guineensis* Jacq.) 635 clone

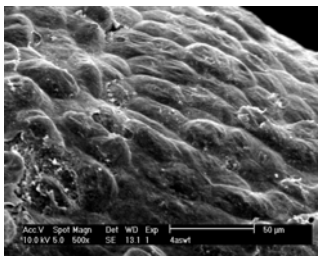


Figure 4. Protoderm, which will become epidermis

In Figure 5, it showed tubular wax crystal on the globular embryo of oil palm. The same structure was found on the Arabidopsis stem (Chen *et al.*, 2003). The formation of cuticular layer occured on the early stage of zygotic embryogenesis as well as somatic embryogenesis. The function of cuticular layer in zygotic embryo is to inhibit osmotic flow of water from liquid endosperm into embryo (Sterk *et al.*, 1991 *in* Mariani *et al.*, 1999), whereas in the somatic embryo, cuticular layer act as a layer to protect the embryo.

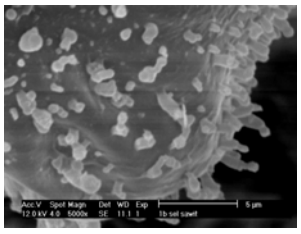


Figure 5. Tubular wax crystal on the surface of oil palm globular embryo

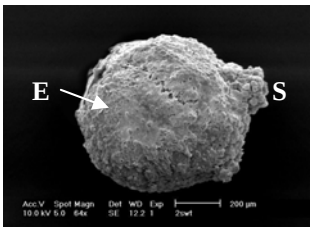


Figure 6. Globular somatic embryo with a suspensor . E=Embryo; S=Suspensor

3.2 Suspensor

In this study, we also found globular embryo with a structure called suspensor (Figure 6). The function of suspensor especially in the early stage of embryogenesis is for nutrition transportation for the embryo (Steeves, 1989). Suspensor can be unicellular or multicellular, in the form of filamentous, round, or irregular.

Figure 7 showed multicellular suspensor with irregular form. It is assumed that this suspensor was inhibited in growth so that it did not function as a normal suspensor. According to Yeung (1993), the degeneration of suspensor has a relation with the

supply of nutririon for embryo development. In rich nutrition environment, usually the suspensor did not develop. In this study, the embryo grew in rich nutrition environment, so that the suspensor did not develop.

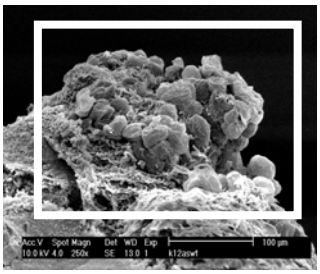


Figure 7. Multicellular suspensor on the globular somatic embryo of oil palm

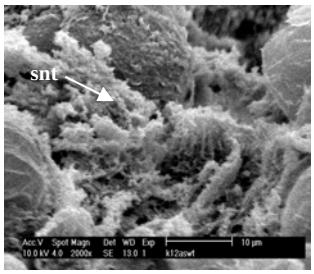


Figure 8. Structure on the cell wall surface of suspensor. snt=supraembryonic network.

It is assumed that the suspensor in this study was inhibited in growth so that it can be predicted that the suspensor in Fig. 7 was still in the proembryo stage. This assumption was supported by the study of Samaj *et al*. (1995). He found extracellular matrix structure in the suspensor of *Pinus nigra* somatic embryo. In this study we found snt (supraembryonic network) on the periphery of suspensor surface (Fig. 8). Therefore, we assumed that with the existence of the snt, the suspensor was still in the proembryo stage. Chapman *et al.* (2000) stated that snt structure occured in the stage before protoderm initiation (proembryo stage).

From the result and discussion, we can conclude several points as follow :

1. The stages of cel wall formation on the proembryo cell of oil palm (*Elaeis guineensis* Jacq.) :
   a. Old cell wall
   b. Pre-secretion of extracellular matrix in the form of cellulose microfibril
   c. Post-secretion of extracellular matrix in the form of cellulose microfibril
   d. New cell wall
2. Extracellular matrix between cell wall of the proembryo was *snt* (supraembryonic network).
3. Protoderm and wax crystal were formed on the oil palm (*Elaeis guineensis* Jacq.) globular somatic embryos. These were the important characteristics leading to the epidemis differentiation.
4. There was undeveloped suspensor and still retained *snt* (supraembryonic network) structure on the globular somatic embryo of oil palm.

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