

# Effect of Glutamine on Storage Protein Accumulation during Maturation of Oil Palm Somatic Embryos (*Elaeis guineensis* Jacq.) Observed by Transmission Electron Microscopy

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**ABSTRACT---** *Oil palm (Elaeis guineensis Jacq.) is the highest oil producing plant that has an important role in increasing our foreign exchange. Oil palm micro propagation via somatic embryogenesis (SE) offers the potential to enhance productivity of oil palm. However, in SE low percentage of germination rates still occurred. It was assumed that it is related to the maturation process. Therefore, this study aimed to identify the effect of glutamine on storage protein accumulation during the maturation of oil palm somatic embryos and to determine optimum concentration of glutamine in accumulating storage protein of oil palm somatic embryos. After 6 weeks of culture in MM I, volume of the somatic embryos became higher and the colour become yellowish. Generally, the enhancement rate of fresh weight both control embryo and treated embryo were not significantly different up to 5 weeks of culture but significantly different on 6 weeks of culture. Analysis of One-way ANOVA and LSD-Multiple comparison showed that enhancement of the fresh weight of control embryos were significantly different with treated embryos on 30 mM glutamine ( $P \leq 0.05$ ). Ultrastructure analysis showed that cell of the mature embryos containing cytoplasm, small vacuoles, organelles and storage protein, which occurred as discrete protein bodies. It was concluded that glutamine contributed to storage protein accumulation and this accumulation was optimum in 30mM glutamine.*

**Keywords---** maturation, glutamine, storage protein, oil palm

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## 1. INTRODUCTION

Plant tissue culture method has been known to propagate oil palm in relatively short time (Handro, 2001). One of the tissue culture method is somatic embryogenesis.

Somatic embryogenesis is a process of embryo formation from a single cell and could germinate to form intact plant (Ignacimuthu, 1997).

Somatic embryogenesis method has many advantages for mass propagation, genetic transformation and production of synthetic seed (Hartmann *et al.*, 1997). However, there is still one aspect that should be improved during the process, namely maturation of somatic embryos. In somatic embryos, the vigour of germinated embryos is low. Therefore, the germinated somatic embryos are weak. (Winkelmann *et al.*, 2004; Seyring and Hohe, 2005 dalam Schmidt, 2006).

It was supposed that the somatic embryos did not undergo maturation process (immature embryos). To overcome that problem, the somatic embryos should enter maturation process by adding arginine and glutamine onto the medium. Therefore, storage protein could be formed and dry weight of embryos could be increased. Therefore, the purpose of this study were to observe the storage protein by transmission electron microscopy and to evaluate the optimum concentration of glutamine for maturation process.

## 2. MATERIAL AND METHOD

### Material

Embriogenic callus of oil palm 638 clone from Marihat, North Sumatra, Indonesia.  
The medium used in this study was Touchet Medium.

### Method

Prematuration stage of somatic embryo

Firstly, the embryogenic callus was cultured in Suspension Initiation medium containing  $2, 26, 10^{-4}$  M 2,4 D dan BAP  $4,44 \cdot 10^{-6}$  M for 3 weeks. Then, the globular somatic embryos were cultured into development embryo medium I without hormone for 1 month. Subsequently, the somatic embryos were transferred onto development embryo medium II containing 5  $\mu$ M BAP for 1 week.

#### Maturation of somatic embryo

The somatic embryos in development embryo medium II were transferred onto maturation medium containing glutamine 10 mM, 20, mM, 30 mM and control (0 mM), All medium supplemented with arginine 5 mM.

This experiment was repeated 3 times. Each time used 10 somatic embryos. The somatic embryos were incubated in the room temperature and 272 lux light intensity for 3 weeks.

#### Measurement of fresh weight

Measurement of fresh weight of somatic embryos was performed every week for 6 weeks. As initial weight, a petri dish was weighed. Then, 10 somatic embryos were put onto the weighed petri dish and weighed as final weight. For average weight the final weight – initial weight and divided by 10. Thereafter, the data was analyzed into graph to compare growth of embryo of every treatment and control.

#### Ultrastructure observation

For ultrastructure observation, the somatic embryos on MM1 (Maturation medium I) supplemented by 20 mM glutamine and was incubated for 3 weeks were used.

Samples were prefixed in 0.1 M cacodylate buffer pH 7.2 containing 5% glutaraldehyde for 24 hr rinsed 3 times in the same buffer, postfixed in 2% osmium tetroxide in cacodylate buffer for 12 hr, rinsed in the same buffer once and distilled water twice, and gradually in an alcohol series, all at 4°C.

The samples were then infiltrated and embedded with Spurr's resin at room temperature. The embedded samples were polymerized in an oven at 70°C for 24 hr.

Ultrathin sections were made with an ultramicrotome at a thickness of 70-90 nm. These sections were stained using aqueous 2% uranyl acetate for 30 min, and with lead citrate for 10 min.

#### Statistical analysis

Statistical analysis was used by *One-Way ANOVA LSD-Multiple Comparison* program SPSS 13.0.

### 3. RESULT AND DISCUSSION

#### Visual observation

Visual observation is needed because it could reveal biological process during growth and development (Nava *et al.*, 2000 in Herman, 2000). Based on visual observation, figure 1 and 2 showed the differences between the somatic embryos after 6 weeks of culture and 0 week of culture in MM1 medium.

Embryos treated with 10, 20 and 30 mM of glutamine grew faster than control. Therefore they were bigger than control (Figure 2). It indicated that glutamine affected the growth of somatic embryos significantly.

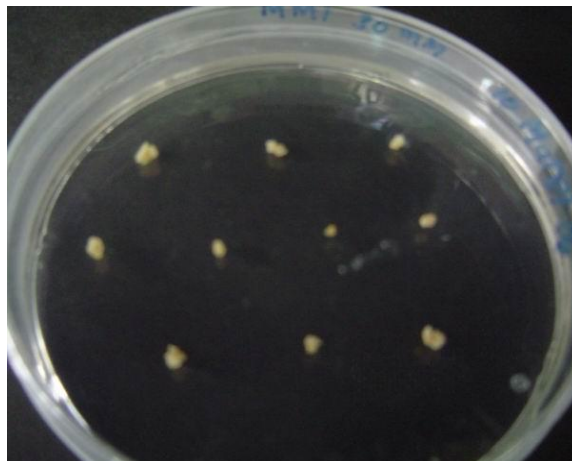


Figure 1. Somatic embryos of oil palm 0 week of culture

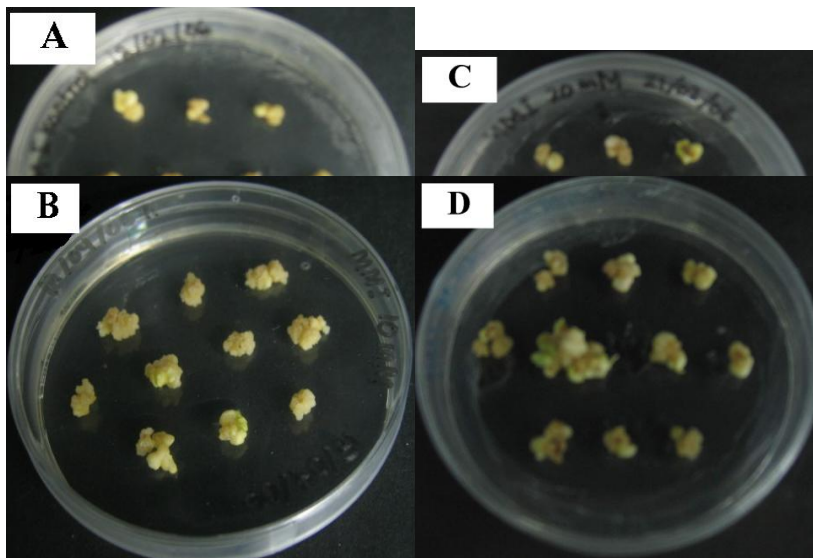
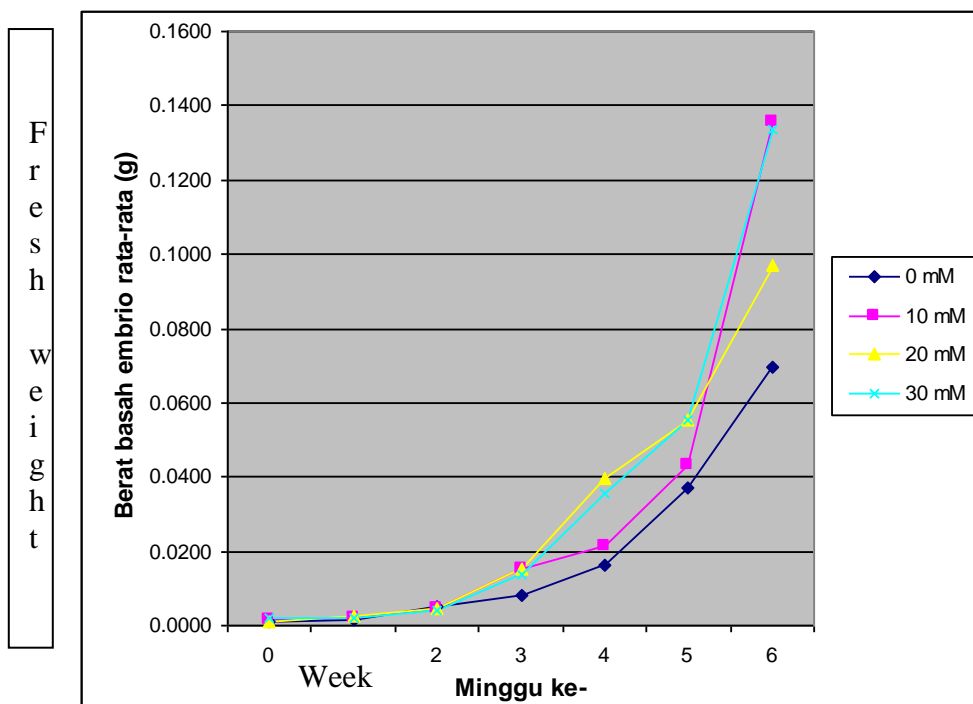


Fig.2. Somatic embryos of oil palm after 6 weeks of culture

- Note :
- A. Somatic embryos (control)
  - B. Somatic embryos in 10 mM MM1
  - C. Somatic embryos in 20 mM MM1
  - D. Somatic embryos in 30 mM MM1

Besides volume changes, colour changes of somatic embryo could become a good parameter to estimate embryo differentiation and predict embryos quality (Nava *et al.*, 2000 , reviewed in Herman, 2000). The observation showed that the creamy colour change into green yellow. It reflected that there was high metabolism activity inside the somatic embryos (Mandal, 2000).

Based on fresh weight average of embryos within 6 weeks, the fresh weight of embryos was increasing. Figure 3 showed that the fresh weight of embryos in 10 mM, 20 mM and 30 mM glutamine increased significantly every week rather than the control.



Note : Y axis is average fresh weight (g)

The increment of fresh weight was assumed that there was accumulation of storage protein. Ogita et al (2000) also stated that the addition of glutamine in embryogenic culture of sugi (*Cryptomeria japonica*) resulted in the increment of dry weight. According to Morcillo et al. (1999), the accumulation of storage protein was important for conversion of the embryos into plantlets. This due to glutamine is a main source of Nitrogen needed for biosynthesis of others amino acid, namely nucleic acid, polyamine, and chlorophyll ((Rodriguez *et al.*, 2006).

In this study we also added arginine into the medium. According to Morcillo (1999), combination of arginine and glutamine was very important in synthesizing storage protein. This is because the arginine and glutamine have a function to regulate transcription process and translation of storage protein expression.

### Observation of ultrastructure

Observation of ultrastructure on mature somatic embryo showed that there were storage protein in the cytoplasm. Storage protein initially synthesized in a rough endoplasmic reticulum and was stored in a protein body (Mandal *et al.*, 2000). Figure 4 showed the protein that was synthesized by endoplasmic reticulum ultrastructurally. Figure 5 shows the diagram of protein bodies formation. Part A shows that the ER (Endoplasmic reticulum) synthesized protein bodies. The process was the same as in figure 4.

All storage proteins are initially synthesized on the rough ER (Bollini and Chrispeels, 1979; reviewed in Chrispeels, 1991). This membrane system consists of an extensive, interconnected network of tubules and cisternae (reviewed in Staehelin, 1997) and serves as the port of entry for secretory and membrane proteins.

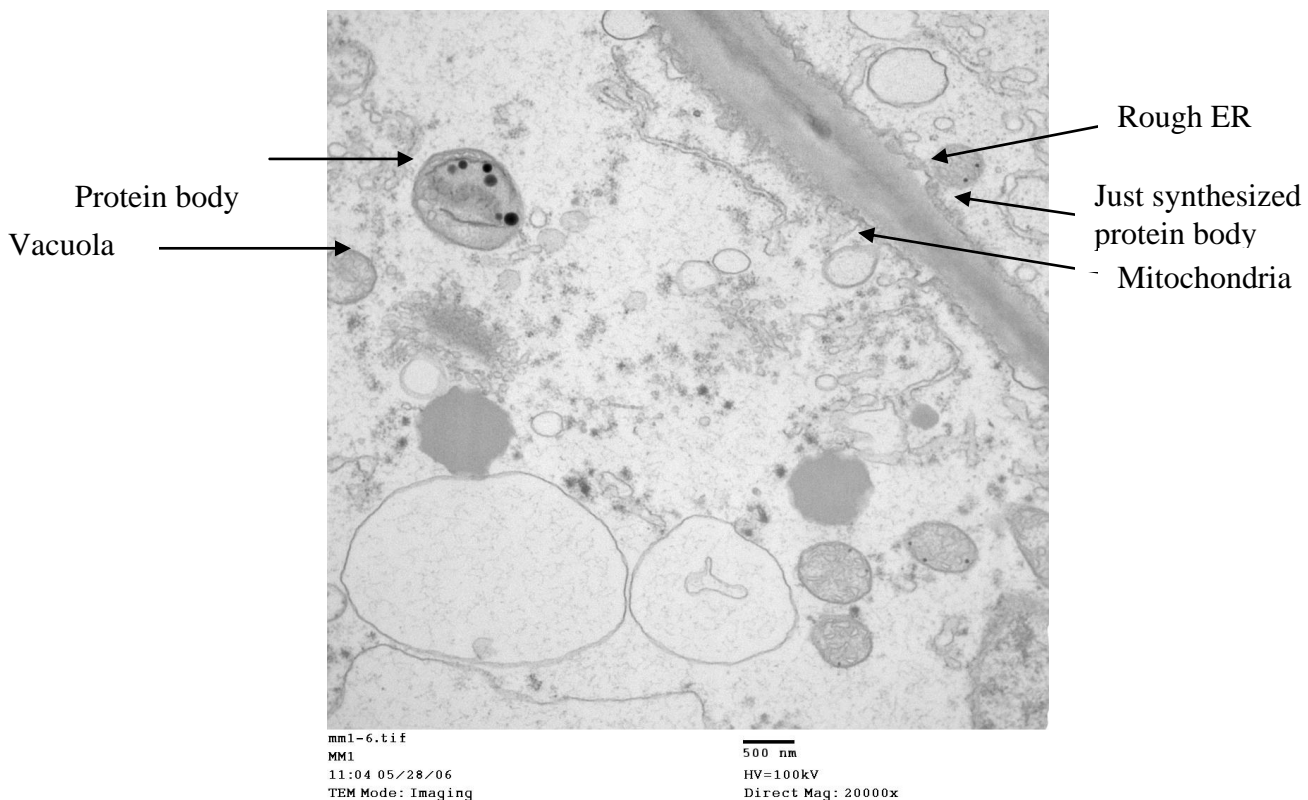


Figure 4. Protein was formed in mature oil palm somatic embryo. Note: ER = endoplasmic reticulum

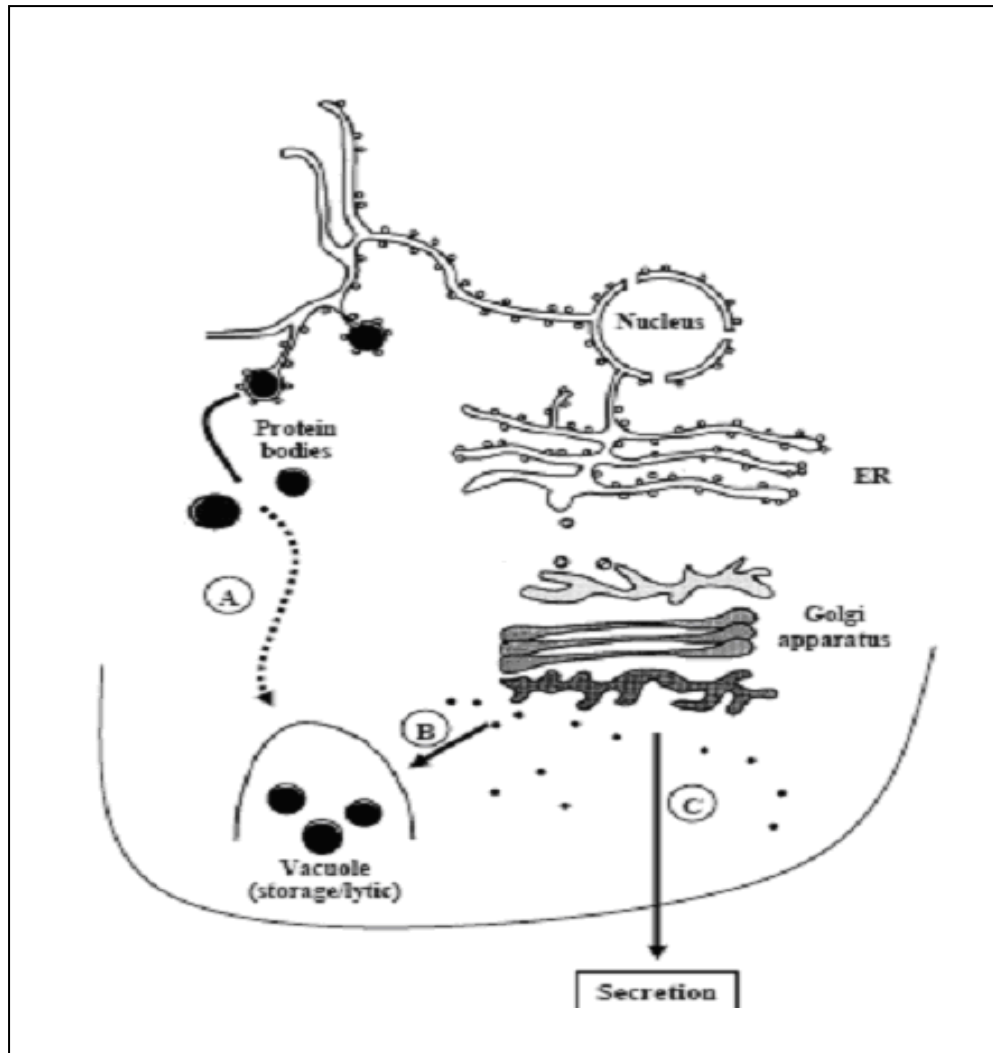


Figure 5. Process of protein bodies formation (Marcel *et al.*, 2005)

Note : Part A shows that the ER (Endoplasmic reticulum) synthesized protein bodies

Based on the result and discussion, we conclude that glutamine induced storage protein accumulation. The optimum concentration of glutamine was 30 mM.

#### 4. ACKNOWLEDEMENT

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#### 5. REFERENCES

- Bollini R., Chrispeels M.J. 1979. The rough endoplasmic reticulum is the site of reserve-protein synthesis in developing *Phaseolus vulgaris* cotyledons. *Planta* 146, 487–501
- Chrispeels M.J. 1991. Sorting of proteins in the secretory system. *Annu.Rev.Plant Physiol. Plant Mol. Biol.* 42, 21–53
- Handro, Walter and Floh, E.I.S. 2001. Neo-formation of flower buds and other morphogenetic respons in tissue cultures of *Melia azedarach*. *Plant Cell, Tissue and Organ Culture* 64: 73-76.
- Hartman, T.H. et al. 1997. *Plant Propagation. Principles and Practice*. Prentice-Hall. Inc. New Jersey.

- Herman, E.B. 2000. *Recent advances in plant tissue culture VII*. Agritech Consultant, Inc. Shrub Oak.
- Ignacimuthu. 1997. *Plant biotechnology*. Science Publisher, Inc. New Hampshire.
- Mandal, S. and Mandal, R.K. 2000. Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. *Current Science*, vol.79, 576-587.
- Marcel, S. and Frankreich, L.M. 2005. Comparative study of the subcellular localisation and the N-glycosylation patterns of a recombinant glycoprotein expressed in *Oryza sativa*, *Nicotiana tabacum*, and *Medicago truncatula*.
- Morcillo, F., Hartmann, C., Duval, Y., Tregear, J.W. 2001. Regulation of 7S globulin gene expression in zygotic and somatic embryo of oil palm. *Physiologia Plantarum* 112: 233-243.
- Ogita, S., Sasamoto, H., Yeung, E.C., Thorpe, T.A. 2001. The Effect of glutamine on the maintenance of embryogenic culture of *Cryptomeria japonica*. *The Plant Cell*. 37: 268-273
- Rodriguez, M.J.P., Suarez, M.F., Heredia, R., Avila, C., Breton, D., Trontin, J.F., Filonova, L., Bozhkov, P., Arnold, S.V., Harvengt, L., Canovas, F.M. 2006. Expression patterns of two glutamine synthetase genes in zygotic and somatic pine embryos support specific roles in nitrogen metabolism during embryogenesis. *New Phytologist*.169: 35-44.
- Schmidt, T., Ewald, A., Seyring, M., Hohe, A. 2006. Comparative analysis of cell cycle events in zygotic and somatic embryos of *Cyclamen persicum* indicates strong resemblance of somatic embryos to recalcitrant seeds. *Plant Cell*. 67: 131-136.
- Staehelin L.A. 1997. The plant ER: A dynamic organelle composed of large number of discrete functional domains. *Plant J*. 11, 1151–1165