Effect of Glutamine Addition in Maturation Stage on the Germination and Plantlet Conversion of Oil Palm (*Elaeis Guineensis Jacq.*) Somatic Embryo

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ABSTRACT--- ***The enhancement of oil palm productivity due to increasing of world demand should be supported by stocks of good quality seedling. Besides from the seed, recently the oil palm seedling has been produced through micropropagation of oil palm. The method of oil palm somatic embryogenesis could provide seedling in large quantity with minimum genetic variation. However, there is still a problem in embrio germination and plantlet conversion due to immature somatic embryo. In this study, we performed evaluation of the effect of glutamine amino acid in maturation stage on germination and plantlet conversion of somatic embryo. In addition, we also conducted histological evaluation of germinated somatic embryo by using light microscopy. The observation of germination and plantlet conversion was performed after incubating on the medium supplemented with glutamine for 3 weeks. The germination medium was without glutamine. The result showed that the highest germination percentage (82.5%) in germination medium for 10 weeks. This result after incubated on the medium supplemented with 10 mM glutamine for 3 weeks. Whereas the conversion into plantlet was achieved on the medium supplemented with 20 mM glutamine.***

Keywords--- oil palm, somatic embryo, glutamine, germination, conversion

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1.INTRODUCTION

Nowadays, oil palm is becoming part of our life. Data from oil world (2010) stated that the cultivation of oil palm in the world reached 12,8 million ha. It is smaller compared to edible oil producing plant, such as soybean (102,4 million ha), rapeseed (31,7 million ha), sun flower (24,6 million ha) dan cotton seed (30,9 million ha). In addition, it is written that oil palm is the highest oil producing plant (3,7 million ton/ha/year) compared to soybean (0.38 million ton/ha/year), rapeseed (0,75 million ton/ha/year), sun flower (0,48 million ton/ha/year). Several daily product from oil palm, namely fry oil, soap, butter, cosmetics, have been widely used (Belgert, 2000). Oil palm also could produce biodiesel (Edem, 2002). The demand of oil palm is increasing as the population increase (Belgert, 2000).

To support the successfulness of oil palm cultivation, recently, the micropropagation of oil palm through somatic embryogenesis has been developed. The advantage of this technique could increase the productivity of the plant up to 20-40% (Subronto dan Asmono, 2000; Latif, 2003 dan 2004; Ginting dan Ginting, 2007) and propagate the clone in high amount within short period.

The material for somatic embryogenesis is young leaf.as a somatic tissue siurce. Due to based on vegetative tissue, the somaclonal variation from the plant could be minimalized. However, the somatic embryogenesis method used produced unsynchronized germination and the plantlet were not vigour. This problem due to the process of somatic embryo maturation was not perfect (Morcillo *et al.*,1999). To solve this problem, in this tudy, we performed the effect of glutamine (amino acid) in maturation stage on the germination and conversion of somatic embryo into plantlet. We also observed the histology of germinated and converted oil palm somatic embryo by light microscopy.

2. MATERIAL AND METHOD

2.1. Material

The material was embryogenic callus derived from young leaf explant. MK 638 clone embryogenic callus was obtained from Indonesia Oil Palm Research Institute, Marihat, Pematang Siantar, Indonesia.

Prior to germination and conversion treatment, the embryogenic callus underwent the following phase (Table 1).

Tabel 1 The phase of somatic embryogenesis in oil palm clone MK 638

No	Somatic embryogenesis phase	Medium	Plant growth regulator	Incubation time
1	Initiation	MIS 4	2,4-D 226 μM; BAP 4,44 μM	4 weeks
2	Development I	MPE I	-	4 weeks
3	Development II	MPE II	BA 5 μM	1 week
4	Maturation I	MM I	Glutamin 0; 10; 20; 30 mM; Arginin 5 mM	3 weeks

2.2. Method

2.2.1. Germination and conversion of somatic embryo

The somatic embryos were cultured on the maturation I medium (MM I) supplemented with 0, 10, 20, 30 mM glutamine for 3 weeks. The matured somatic embryos were then subcultured onto germination medium (GM). The GM was Touchet medium without plant growth regulator. One Petrie of maturation medium consists of 10 embryos. When the mature embryo was subcultured onto germination medium, the 10 embryos were divided into two. Therefore, each Petrie of germination medium consists of 5 embryos. The embryos in GM were incubated for 8 weeks on 25^{o}C and 12 hr photoperiod.

2.2.2.Data analysis of germination and conversion of embryos

The germination and conversion process was observed from 0 week up tp 8th week by recording the amount of root, shoot and plantlet developed from the embryos. The graph was made based on the observation. The statistic analysis was *one way* Anova (p<0,05) and *post hoc test LSD multiple comparison.*

2.2.3. Histological analysis

The histology analysis was made by using light microscopy. Paraffin was used for fixation. The method consists of seceral stage, namely fixation, washing, dehydration, infiltration, embedding, semithin sectioning, putting the semithin sections on the object glass and staining.

3. RESULT AND DISCUSSION

3.1. Maturation of somatic embryo

The growth of embryo could be observed by the increasing of embryo size during cultured in maturation media (Fig. 3.1.). The growth involving the process of cell enlargement and division. It has been known that the enlargement of cell occured due to the increasing of cell volume. The number of vacuoles increased but others organelle did not. The vacuoles in the seed is known as place for storage protein (Herman and Larkin, 1999). It is called *protein-storage vakuola* (PSV) (Jolliffe *et al.*, 2005). This has relation with metabolism process during maturation, namely protein synthesis, oil and starch as nutrition storage needed for the next stage (Dodeman *et al.*, 1997).

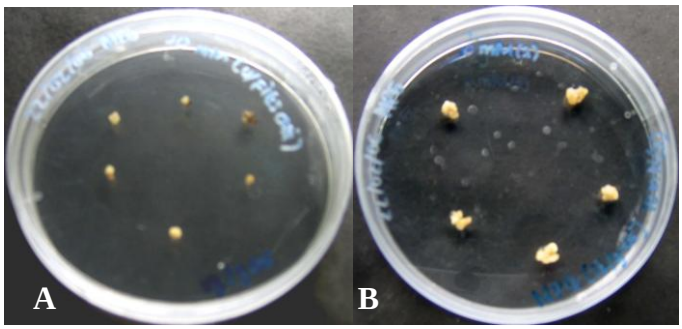

Figure 3.1 The somatic embryos before maturation (A), somatic embryos after 3 weeks of culture in maturation medium (B)

Besides cell enlargement, other form of growth occuring was the division of cell activity. In this study, the division of cell shown by the formation of secondary embryo. Das Neves et al. (1999) stated that it was due to embryogenesis process. The meristematic competent and cytokinin determined the process (Mähönen, 2005). The secondary somatic embryo could be separated from the primary embryo. They could germinate and developed into plantlet.

3.2. Germination and conversion of somatic embryos

There were four responses of embryos in germination medium (Fig. 3.2.). First, embryo formed root only. Second, the germination of embryo was shoot only. Third, Embryo developed into shoot and root (plantlet). Four, the embryos did not germinate. Figure 3.3. shows the germination and conversion of somatic embryos.

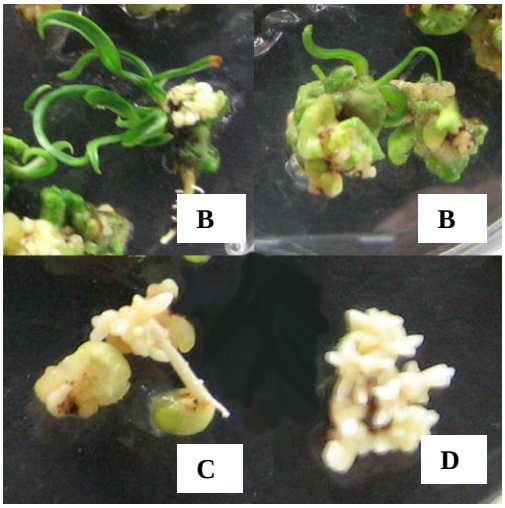

Figure 3.2. Four responses of somatic embryos to the glutamine Plantlet conversion(A), shoot germination only (B), Root germination only (C), and did not germinate (D), (note: s=shoot, r=root, e=embrio).

3.3. Morphogenesis of shoot, root and plantlet

Fig. 3.3. shows that the highest percentage of shoot germination (67.5%) was on the 30 mM glutamine. Whereas the highest percentage of root germination (10%) was on 20 mM glutamine. The highest percentage of plantlet conversion (27.5%) was on 20 mM. The various response occurred during somatic embryogenesis of oil palm due to internal factor, namely nutrition and hormone involved in cell division, elongation, polarity, differentiation and external factor, such as light and environment (Dodeman *et al.* 1997).

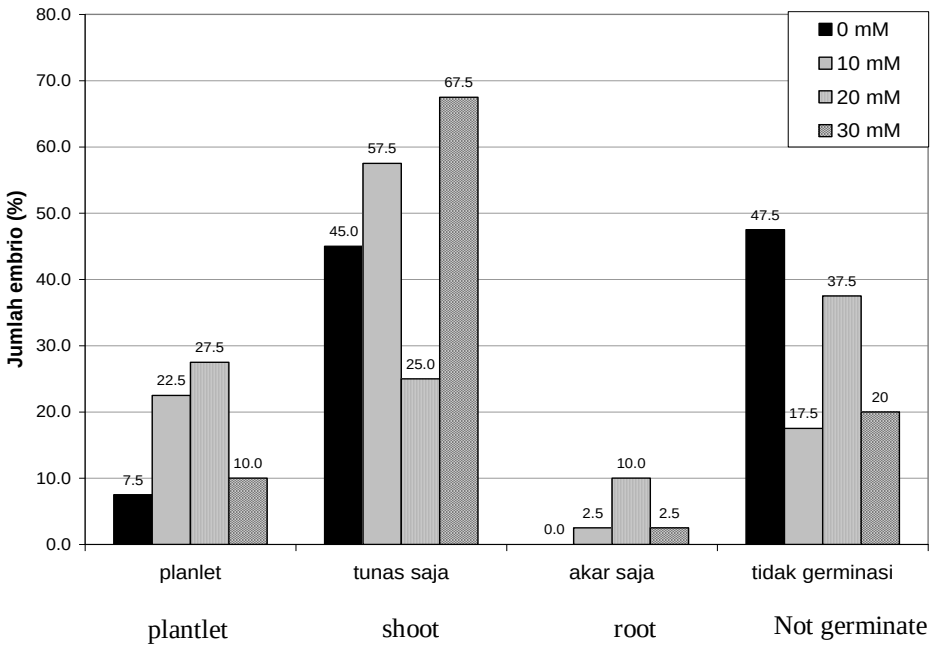

Figure 3.3. Shoot and root germination, plantlet conversion of oil palm somatic embryos 8 weeks of culture on germination media

Besides nutrition, hormonal factor influenced the development of root. According to Soh *et al.* (1999), auxin could affect initiation and development of root primordium. The percentage of root germination was low on 30 mM glutamine. This could be caused by the low synthesis of endogenous auxin (Hamasaki, 2005). Therefore, it could be assumed that

the inhibition of root germination due to in the form of inactive auxin, IAA-Glu (conjugation of IAA and glutamine) (Woodward dan Bartel, 2005).

3.4. Plantlet conversion

The successful of plantlet conversion shows that there was balance interaction between auxin and cytokinin hormones (Soh *et al.*,1999). It was also determined by the complete maturation process of somatic embryo (Morcillo *et al*., 1999). In the maturation process, storage protein was deposited and used for plantlet conversion.

3.5. Histology of oil palm somatic embryo in conversion stage

The observation of somatic embryo was by light microscopy. Figure 3.4. shows the converted somatic embryo. It consists of shoot apical meristem, root apical meristem and vascular bundle that connect shoot and root apical meristem. The occurence of vascular bundle that connect shoot and root apical meristem was the characteristic of somatic embryo.

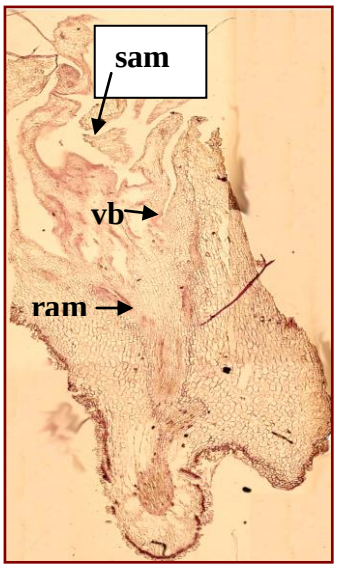

Figure 3.4. Histology of converted oil palm somatic embryo. Note: sam (shoot apical meristem), ram (root apical meristem), vb (vascular bundle that connect sam and ram)

A research of second maturation of oil palm somatic embryo by using abscisic acid was conducted and a paper is being written.

4. CONCLUSION

Based on the result and discussion, we could conclude as follows :

1. The addition of 20 mM glutamine in maturation medium could support the development of somatic embryo into plantlet.
2. Histologically, the converted somatic embryo consists of shoot apical meristem, root apical meristem, vascular bundle that connect shoot and root apical meristem.

5. ACKNOWLEDGEMENT

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