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Effect of Abscisic Acid and Desiccation on Germination and Conversion of Oil Palm (*Elaeis guineensis* Jacq.) Somatic Embryos in Medium Supplemented with Gibberellic Acid (GA_3)

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ABSTRACT---- ***Oil palm is a commodity plant which has a great potential to develop due to a high economical value. Palm oil is useful for food industry, detergent and soap industry, cosmetic, and as alternative fuel (palm biodiesel). However, oil palm plantation in Indonesia mostly derived from seed. Supply of seeds conventionally is not sufficient for plant that will be developed widely. Micropropagation via somatic embryogenesis technique can produce high yield clones in a short period. However, low level and unsynchronous germination, precocious germination, and also inefficient conversion are the main problems in somatic embryogenesis. The objectives of this research were: to evaluate the effect of abscisic acid (ABA) and desiccation on germination and conversion of somatic embryos, to determine the optimum concentration of ABA and desiccation duration in obtaining the synchronous germination and high conversion level, and to evaluate the histology of somatic embryo in germination and conversion stage observed by light microscope. The research material was calli of oil palm 638 clone. Suspension culture of oil palm was initiated by inoculating 0,5 g of the calli into suspension initiated medium supplemented with 226 µM of 2,4-D and 4,44 µM of BAP and cultured for 4 weeks. Subsequently, the somatic embryos were developed into embryo development medium I without plant growth regulator for 4 weeks. Thereafter, the developed somatic embryos were plated on embryo development medium II supplemented with 5 µM BA for 1 week. Then, the somatic embryos were subcultured into maturation medium I supplemented with 20 mM glutamine and 5 mM arginine cultured for 3 weeks. Finally, the mature somatic embryos were subcultured into the maturation medium II supplemented with the various concentration of ABA (0, 10, 25, and 50 µM) and cultured for 2 weeks and desiccated for 2, 3, and 4 hours. The complete mature somatic embryos were subcultured into pregermination medium supplemented with 100 µM*** GA_3 ***to induce the germination. The germinating somatic embryos were then subcultured into the germination medium without plant growth regulator. After 10 weeks of culture, germination and conversion percentage were counted and analyzed by non parametric statistic test of Kruskal-Wallis. The highest germination percentage was on the embryos treated with 25 µM ABA (100%). The highest conversion percentage of somatic embryos into plantlet was on the embryos treated with ABA 25 µM (75%). These results are significantly different with two other concentrations (10 µM and 50 µM) treatment. Desiccation duration of 2, 3, and 4 hours were optimum duration range to increase germination and embryo conversion into plantlet because more than 80% embryos germinated and more than 60% embryos converted into plantlet. Converted somatic embryos have vascular bundle connecting shoot apical meristem and root apical meristem. It can be concluded that the supplementation of ABA and desiccation treatment could increase the germination and conversion of somatic embryo into plantlet synchronously.***

Keywords--- germination, conversion, abscisic acid, desiccation, somatic embryo, oil palm.

1. INTRODUCTION

Oil of oil palm is good because non cholesterol.It also contains caroteen as anti cancer and tocopherol as source of vitamin E (Fauzi *et al*., 2005). Since this plant is very valuable, it is needed to propagate it by tissue culture method.

There are 2 kinds of propagation method, namely organogenesis and somatic embryogenesis. If we use organogenesis method, we should transfer the shoot onto rooting medium. Therefore, it is quite laborious and expensive. By somatic embryogenesis method, we could obtain plantlet from the embryo. Consequently, we need not to transfer it to the rooting medium. This way, it is cheaper and easier.

Mariani et al. (2014) has performed maturation of somatic embryos of oil palm by adding glutamine into the medium. There was increasing of germinated embryos. However, we would like to increase more up t0 90-100% germination rate of oil palm somatic embryos. To achieve it, the abscisic acid (ABA) was added into the medium. The purpose is to achieve final maturation of somatic embryos. By adding the ABA, somatic embryos will dessicate as normal seed when in dormancy period.

After underwent the dessication process, it is necessary to add giberellic acid. By adding the giberellic acid, the activity of ABA will be decreased and the embryo could germinate normally.

Based on the background above, the purpose of this study were as follows:

1. To evaluate the effect of ABA on the germination of somatic embryos
2. To observe the structure of germinated somatic embryo by light microscopy

2. MATERIAL AND METHOD

2.1 Material

The material of this study was the somatic embryos of oil palm that have been underwent the following process :

Table 1. The stages of somatic embryogenesis in oil palm clone 638

No	Stages of somatic embryogenesis	Medium	Plant growth regulator or supplemen	Incubation time
1	Induction	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 20 mM; arginin 5 mM	3 weeks
5	Maturation II	MM II	ABA 0; 10; 25; 50 µM	2 weeks

After culturing in MM II, the somatic embryos were dessicated for 2,3, and 4 hr (Figure 1).

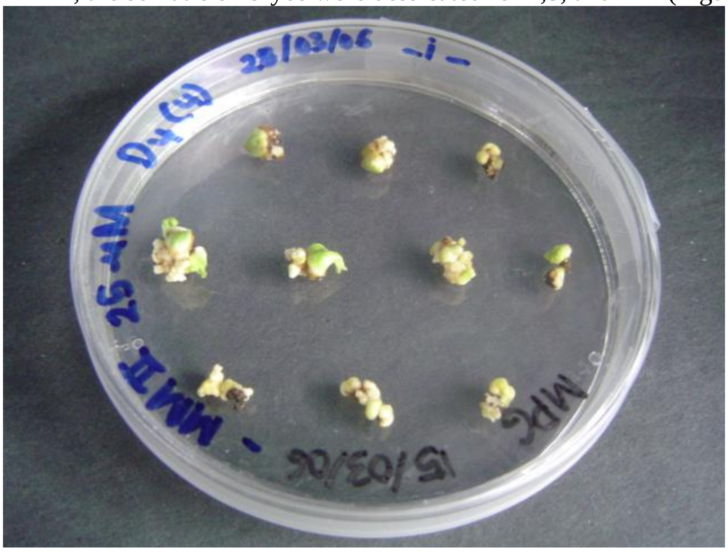

Figure 1. Oil palm somatic embryos during dessication process

2.2 Methods

2.2.1 Germination medium

For observing the development of shoot and root, the dessicated somatic embryos were cultured on germination medium. There were 2 kinds of germination, namely pre-germination medium (MPG) and germination medium without hormone (MTH) (Tabel 2).

Tabel 3. Composition of germination medium

Medium	Basic medium	Hormone	Sucrose	Gelling agent
MPG	Touchet	GA_3 100 µM	3 %	Phytagel 0.25 %
MTH	Touchet	-	3 %	Phytagel 0.25 %

2.2.2 Subculture

The somatic embryos were subcultured onto MPG medium. One petri dish contain 5 somatic embryos. The condition of culture was 27^{o}C and 12 hr photoperiod for 10 weeks. The somatic embryos with shoots were then subcultured onto MTH medium. In this medium, the shoot and root developed.

2.2.3 Quantitative analysis

Plantlet was counted from the culture after 10 weeks in medium. The statistic anaysis was Kruskal-Wallis and *multiple comparison* Post Hoc Tamhane's T2. The purpose was to analzed the data quantitatively so that the optimum ABA concentration and dessication duration would be known.

2.2.4 Observation by light microscopy

The germinated somatic embryos were fixed by paraffin method. Then, semithin sections were made and stained with Mayer's Hemalum. Thereafter the semisectioned samples were observed by light microscopy.

3. RESULT AND DISCUSSION

3.1. Effect of Abscisic acid (ABA) supplementation on germination and concersion of oil palm somatic embryos.

The development and morphology of somatic embryos are similar to those of zygotic embryogenesis. However, somatic embryos lack of dessication and dormancy period. The somatic embryo directly develop into plantlet (Zimmerman, 1993; Ziv, 1999).

Due to the lack of dessication and dormancy, the somatic embryos often undergo early germination. Therefore, the plantlet is not vigour (Ziv, 1999). To overcome this, we added ABA so that the somatic embryos entering dessication process and dormancy. Consequently, the early germination could be avoided.

Germination is a process initiated by the imbibition of water and development of radicula (Bewley, 1997; Wareing & Phillips, 1981). After germination occuring, embryo will develop into plantlet that have shoot and root (Bewley, 1997; Anonim, 2005). This process is called conversion.

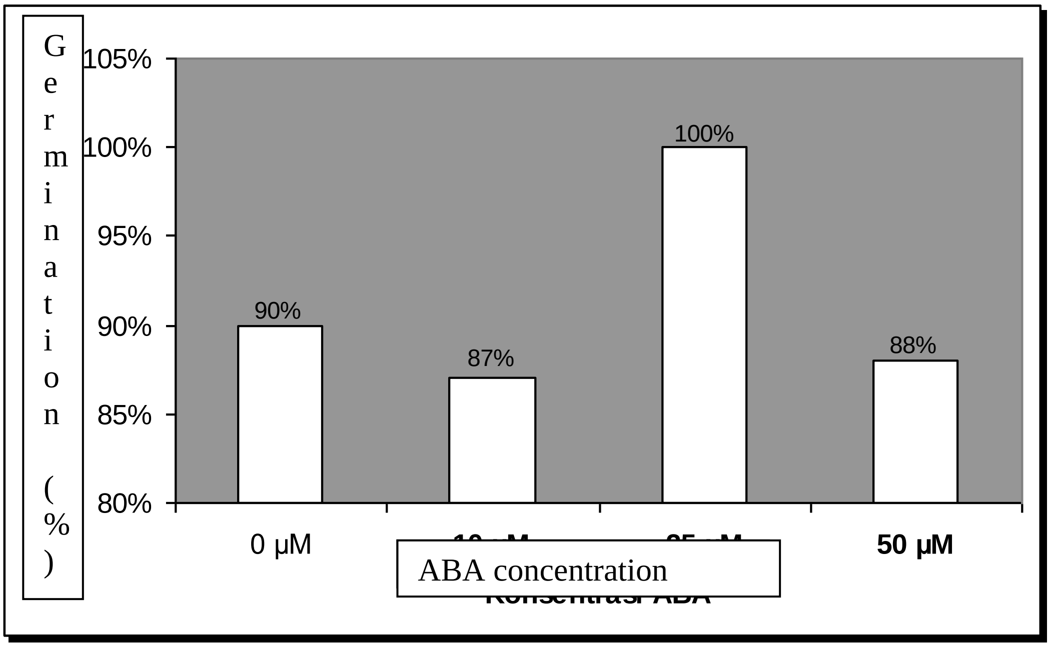

Figure 1. Effect of abscisic acid on the percentage of oil palm somatic embryo germination.

The figure 1 shows that the somatic embryos treated with 25 µM ABA could achieve 100% germination. Data analysis with Kruskal-Wallis ($p<0,05$) stated that the somatic embryos germinated in 4 concentration of abscisic acid treatment, significantly different. This result supported the result of Bertossi *et al*. (2001). Bertossi reported that their study with Abscisic treatment resulted in development of shoot. In this study, the somatic embryos developed into plantlet because we added gibberelin (GA3) in germination medium.

GA3 could reduce level or effect of abscisic acid in embryo (Ogawa *et al*., 2003). GA3 was needed to stop dormancy period induced by abscisic acid so that the embryos could germinate normally (Bentsink & Koornneef, 2002).

The somatic embryos that did not treated with ABA (control) underwent early germination (Figure 2). It means that the somatic embryos did not dessicate and dormant. They directly germinate but abnormal.

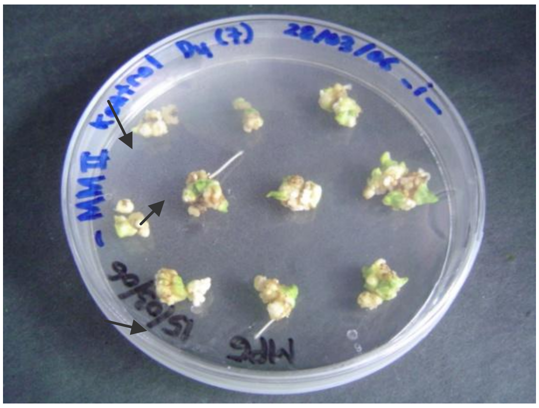

Figure 2. The somatic embryof of oil palm in control medium (without abscisic treatment) underwent early germination (only root, as shown by arrow).

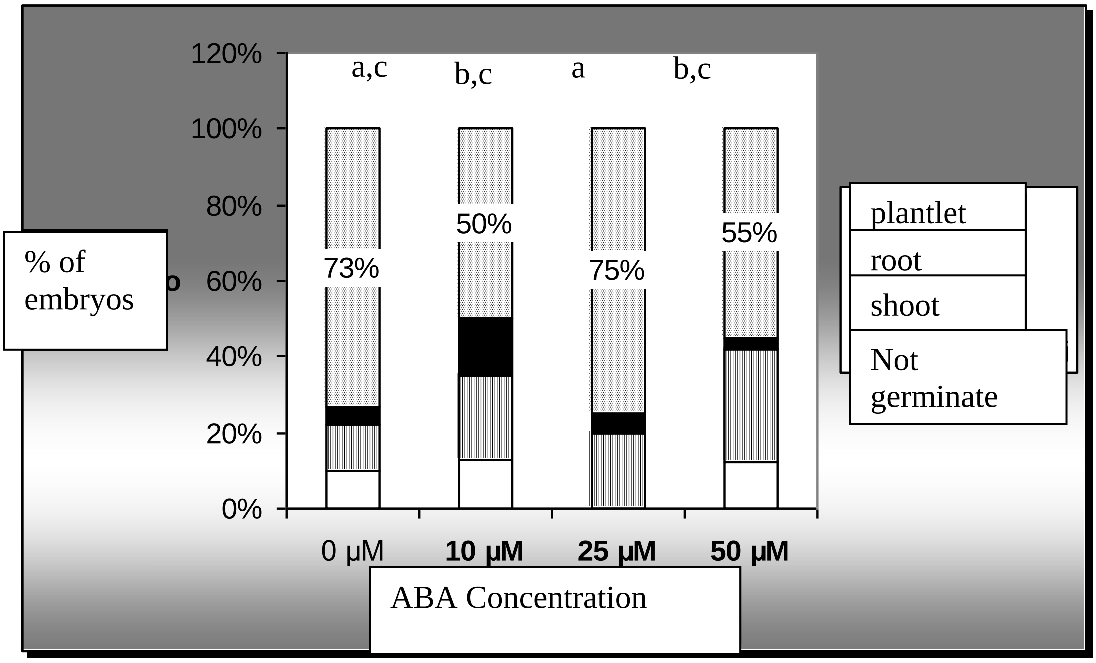

Figure 3. Effect of abscisic acid on the conversion of oil palm somatic embryos

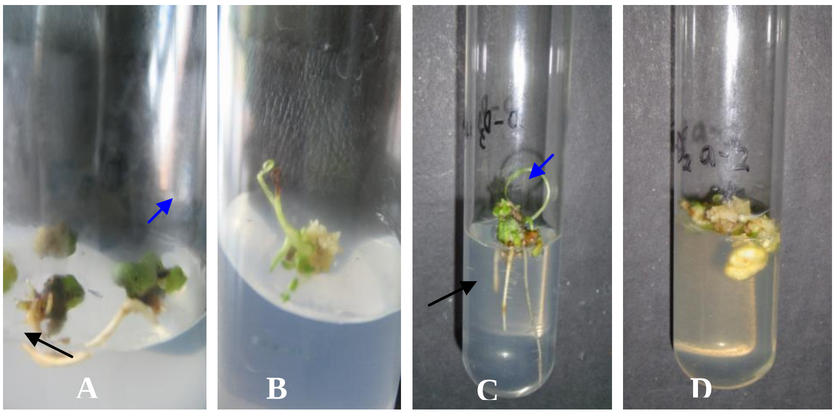

Figure 4. The somatic embryos developed into root [A], shoot [B], shoot and root (plantlet) [C], dan did not germinatei [D] (Note : → = root; → = shoot)

Figure 3 shows the result of observation on the effect of abscisic acid in conversion of oil palm somatic embryos. Conversion is a process of somatic embryo development into shoot and root. We named it plantlet (Anonim, 2005). The control also shows 7#% conversion into plantlet. Perhaps it was due to the addition of glutamine in the previous medium (MM I). Glutamine is an amino acid that helps the formation of storage protein needed for germination. The addition of 25 µM Abscisic acid (ABA) showed the highest conversion into plantlet. Figure 4 shows the development of somatic embryos.

4.2. Effect of dessication treatment on the germination and conversion of oil palm somatic embryos

Desiccation tolerance is one of a fundamental character in the seed. This character appear in the late development of seed, namely in final maturation. Dessication tolerance is important in life cucle of a plant as a strategy for adaption so that the seeds could survive during storage (Leprince *et al*., 1993 dalam Mariani *et al*., 2000). Toleransi desikasi merupakan salah satu sifat yang fundamental pada suatu biji. Sifat ini diperoleh pada perkembangan biji tahap akhir, yakni saat maturasi akhir. Toleransi terhadap desikasi sangat penting dalam penyempurnaan siklus hidup tumbuhan sebagai suatu strategi adaptasi agar biji dapat bertahan hidup selama penyimpanan atau adanya cekaman dari lingkungan serta untuk menjamin persebaran spesies (Leprince *et al*., 1993 dalam Mariani *et al*., 2000). Dessication tolerance in somatic embryo should be induced, by adding the abscisic acid in the medium.

There were 3 variation of dessication tolerance, 2,3, and 4 hrs. Data analysis using statistical analysis Kruskal-Wallis ($p<0,05$) showed that the germination percentage of oil palm somatic embryos in each dessication was not different significantly. Almost all the embryos could germinated in those three variation (Figure 5).

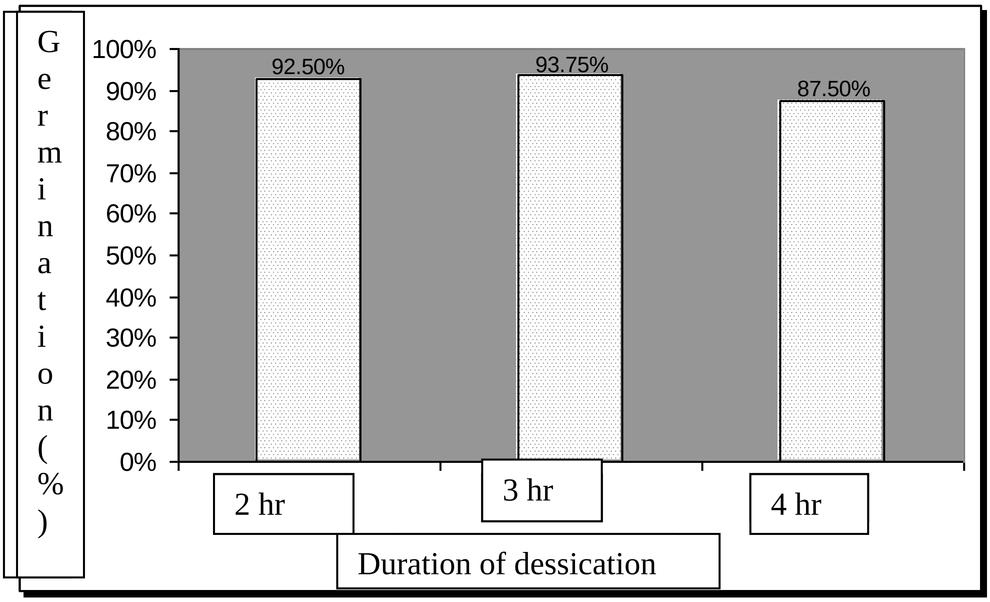

Figure 5. Effect of dessication duration on the germination of oil palm somatic embryos

Figure 5 showed that the somatic embryos could germinated more than 80% in all variation of dessication, 2,3 and 4 hr. Three hr dessication could induce 93.75% germination.

4.3. Histological observation of oil palm somatic embryo in germination and conversin stages by using light microscopy

A goal in somatic embryogenesis pathway is to achieve an efficient regeneration system. If the structure of somatic embryos similar as that of zygotic embryo, the somatic embryogenesis is efficient (Mariani *et al*., 2000). Somatic embryogenesis could form bipolar structure so that it could save time in plant production system.

Figure 6 showed the histology of converted somatic embryo in oil palm. Fig. 6 A indicated the shoot apical meristem and root apical meristem connected by vascular bundle. This is an important character of somatic embryo. Fig. 6 B indicated tracheid in vascular bundle. Tracheid is one of xylem component (Campbell *et al.*, 1999).

Based on the result and discussion, we could conclude that the addition of abscisic acid and dessication treatment could increase the germination and conversion of oil palm somatic embryo. Therefore, the regeneration of oil palm somatic embryo in this study is efficient and could be applied in others plants.

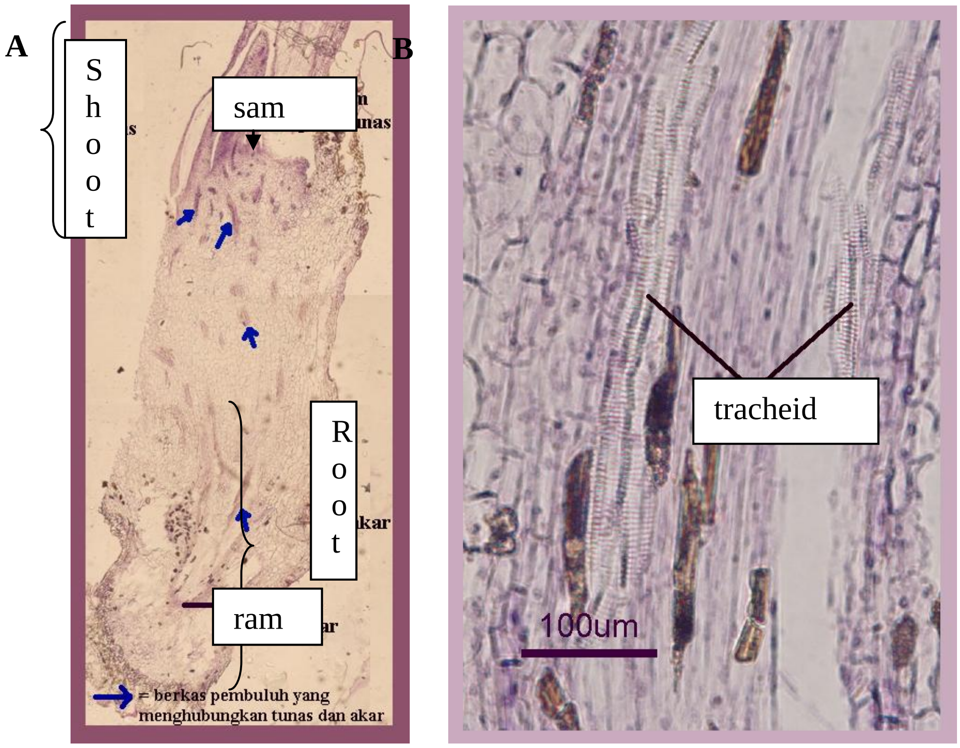

Figure 6. Semithin section of oil palm plantlet with magnification 40x [A] and 200x [B].

Note : sam = shoot apical meristem, ram= root apical meristem

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