Effect of Abscisic Acid (ABA) on Desiccation Tolerance in Maturation Stage of Oil Palm Somatic Embryos

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ABSTRACT--- ***Oil palm is a member of Palmae Family that has an important economic value because its fruit containing very useful oils. The result of oil refinery can produced frying oil, raw materials for margarine and soap manufacture, lubricating material in steel industry, and as an alternative fuel (biodiesel). In Indonesian oil palm is generally cultivated by seed, which is heterozygote. Therefore, it obstructs production enhancement and cause a problem in supplying high yielding clone. Tissue culture technique via somatic embryogenesis is very potential to produce plant in large scale and in a relatively shorter time. In addition, it will reduce somaclonal variation so that the plantlet will be the same as their mother plant. Somatic embryogenesis include induction, development, maturation, germination, and convertion. The crucial problem in maturation of the somatic embryo is embryo does not mature and it only forms shoot in germination. This problem can be overcome by treatment of the somatic embryo using ABA (abscisic acid). ABA is known to be involved in late maturation phase to desiccation tolerance by inducing LEA (late embryogenesis abundant) protein synthesis. Desiccation tolerance is required after the seed mature as a strategy for seed in order to survive in facing environmental stress. The aim of this study was to evaluate the effect of ABA on desiccation tolerance in maturation phase of oil palm somatic embryo. This study used somatic embryo that has been cultured on maturation medium I supplemented with 5 mM arginine and 20 mM glutamine. Subsequently, the somatic embryos were cultured on maturation medium II (MM II) supplemented with ABA 0 μM (control), 10 μM, 25 μM, and 50 μM. After 2 weeks of culture, based on one-way ANOVA ($p < 0.05$), means of embryo fresh weight difference of each treatment group and control did not significantly different, indicating that ABA concentrations in MM II did not significantly affected the embryo fresh weight. On the other hand, ultrastructure observation of embryo by transmission electron microscopy (TEM) showed the accumulation of starch and protein outside the vacuole, which is assumed as LEA protein. Desiccation treatment in three variations times, namely 2 hr, 3 hr, and 4 hr with filter papers resulted in gradually increasing loss of water percentage. For treatment group of 25 μM ABA, loss of water percentage did not increase drastically. Therefore, it was showed the effect of 25 μM ABA that tolerance to desiccation.***

Keywords--- oil palm, somatic embryo, maturation, ABA, dessication treatment

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1.INTRODUCTION

In general, oil palm in Indonesia are propagated by sowing the seed. However, the seed is heterozygot. Therefore, the seed are vegetatively different (Mariani *et al*., 2005). Consequently, there would be a problem in providing seedling and inhibit he enhancement of oil production. To overcome the problem, advance micropropagation is needed, namely somatic embryogenesis.

In somatic embryogenesis, we shoud make the somatic embryos undergo maturation process (Merkle *et al*., 1995). To overcome this problem, the somatic embryos should be matured with ABA treatment. ABA influences the maturation process for dessication by inducing protein synthesis *late embryogenesis abundant* (LEA) (Taiz & Zeiger, 2002). In natural seed, dessication tolerance is needed after the seeds maturing so that they survive when facing environment pressure.

Based on the background, the purpose of this study was to evaluate the effect of ABA on desiccation tolerance in mature somatic embryos and the survival of somatic embryos. In addition, the mature somatic embryos will be used for the material of oil palm synthetic seed.

2.MATERIAL AND METHOD

2.1. Material

The material used in this study is the somatic embryos of oil palm that has been maturing in maturation medium I (MM I) containing 5 mM arginine and 20 mM glutamine for 3 weeks (Mariani et al., 2014).

2.2. Methods

1. Final maturation

The somatic embryos in MM I were subcultured into maturation medium II (MM II) containing ABA 0 µM (control), 10µM, 25 µM, dan 50 µM. Previously, the embryos were weighed (initial fresh weight). The incubation room was 25^{o}C, 12 hrs photoperiod for 2 weeks. selama 2 minggu.

2. Transmission electron microscopy

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^{o}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^{o}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.

3. Desiccation tolerance.

The somatic embryos in MM II were desiccated by using filter paper. There were 3 groups, first group (desiccated for 2 hr). Second group (desiccated for 3 hr), third group (dessicated for 4 hr.). Previously, the somatic embryos were weighed (final gresh weigh). After desiccating, the somatic embryos were weighed (dry weight).

3.RESULT AND DISCUSSION

The visual observation of somatic embryos on MM II medium was increasing the weight and size. The initial size was 1-2 mm and thefinal size is 2-4 mm. The colour of embryos were white and fresh.

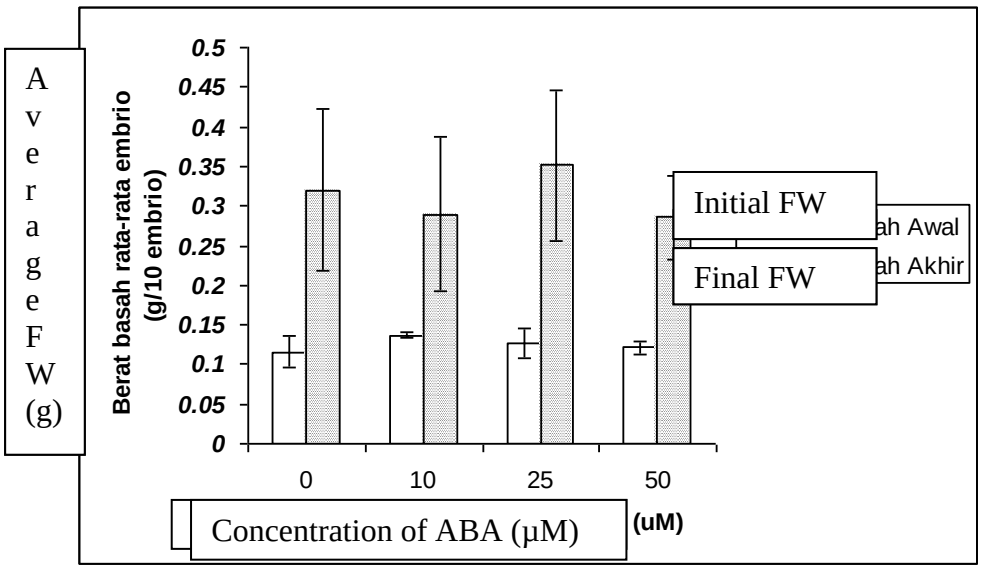

Figure 4.1 The avarage of fresh weight (initial and final) of oil palm somatic embryos on MM II Note : FW = fresh weight

Based on *one-way* ANOVA *Post Hoc Test* LSD ($p < 0.05$), control and the three treatment of ABA shows that avarage fresh weight of embryos were not significantly increased (Fig. 4.1.). It means that the concentration of ABA was notsignificantly effected on final fresh weight.

ABA did not increase the frsh weight of somatic embryos. It is assumed that ABA induced the synthesis of protein during maturation of embryos, namely late embryogenesis abundant (LEA) protein. Figure 4.2. shows protein and starch inside the cell of mature somatic embryos.

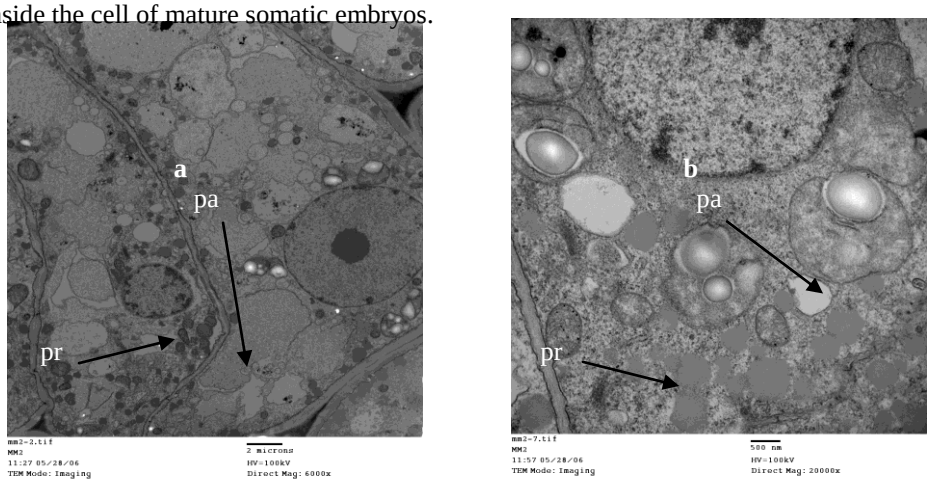

Figure 4.2 Accumulation of starch and protein in mature somatic embryo of oil palm. Note : pa = starch, pr= protein.

Regarding dessication tolerance, control embryo percentage of transpired water 23,85%. In 25 µM ABA, the transpired water increasing but stabil, 27%, 32%, 44% during 2,3,4 hrs respectively. Figure 4.3. shows the transpired water during desiccation. It means that the somatic embryo in 25 µM ABA was desiccation tolerance.

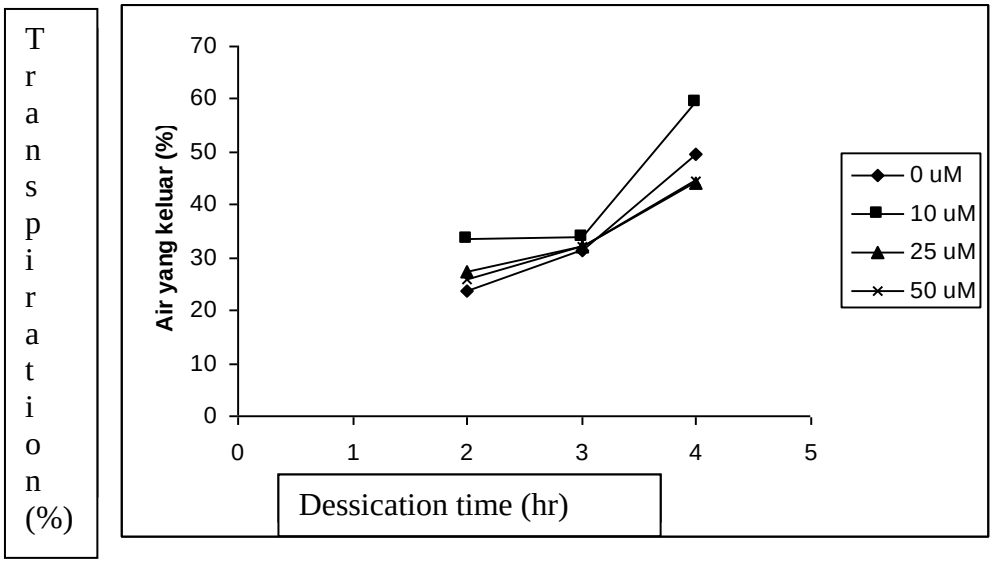

Figure 4.3. Transpired water during dessication treatment in somatic embryos of oil palm.

Bertossi *et al.* (2001) revealed that ABA significantly increasing tolerance of embryo on desiccation speed. In alfalfa somatic embryo, almost 90 to 100% embryos germinated into plantlet after ABA treatment. (Gray & Purohit, 1991).

According to Nedeva & Nikolova (1997), ABA has a function to regulate gene expression for synthesizing LEA protein. The function of LEA protein is to protect seed so that dessication tolerance. In somatic embryo, dessication treatment is applied for avoiding early germination. Therefore, the plantlet became vigour. (Bhojwani & Soh, 1999).

4. CONCLUSION

From the result and discussion, we conclude as follows:

1. The ultrastructure of oil palm somatic embryo in maturation stage showed the accumulation of protein and starch when observed by transmission electron microscopy.
2. The optimum concentration of ABA for desiccation tolerance was 25 µM.

5.REFERENCES

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