

Effect of Plant Growth Regulator on The Growth of Zygotic Embryos in Three Types of Oil Palm Fruit (*Elaeis guineensis* Jacq.) in Tissue Culture

*Pengaruh Zat Pengatur Tumbuh pada Pertumbuhan Embrio Zigotik Tiga Tipe Buah Kelapa Sawit (*Elaeis guineensis* Jacq.) pada Kultur Jaringan*

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ABSTRAK

Kultur embrio adalah cara yang banyak digunakan dan efektif untuk mengatasi sifat dormansi benih di kelapa sawit. Pada tahap perkecambahan, pengaruh regulator pertumbuhan memainkan peran penting meskipun dalam konsentrasi yang relatif lebih rendah di media perkecambahan. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan berbagai konsentrasi zat pengatur tumbuh pada tiga jenis buah kelapa sawit berdasarkan ketebalan cangkang. Penelitian ini dilakukan di Laboratorium Kultur Jaringan PT.Sampoerna Ago Tbk. pada September 2018 hingga Januari 2019. Zat pengatur pertumbuhan yang digunakan dalam penelitian ini adalah NAA, BAP dan GA3. Komposisi zat pengatur tumbuh yang digunakan adalah H1 = 0,05 mg/L NAA + 0,1 mg/L BAP + 0,1 mg/L GA3, H2 = 0,05 mg/L NAA + 0,3 mg/L BAP + 0,1 mg/L GA3, H3 = 0,05 mg/L NAA + 0,5 mg/L BAP + 0,1 mg/L GA3 dan H4 = Tanpa zat pengatur tumbuh. Sedangkan jenis buah kelapa sawit yang digunakan adalah C1 = Dura, C2 = Tenera dan C3 = Pisifera. Penelitian ini menggunakan rancangan acak lengkap faktorial dengan tiga ulangan. Hasil penelitian menunjukkan bahwa secara umum penggunaan zat pengatur tumbuh H1 = 0,05 mg/L NAA + 0,1 mg/L BAP + 0,1 mg/L GA3 dan jenis buah Dura menunjukkan hasil positif berdasarkan parameter viabilitas, skoring, survival dan tinggi *planlet* bila dibandingkan dengan perlakuan lainnya.

Kata kunci: kelapa sawit, in vitro, dura, pisifera, tenera

ABSTRACT

Embryo culture is a widely used and effective way of overcoming the dormancy nature of seeds in oil palm. At the germination stage, the influence of growth regulators play an important role although in relatively lower concentrations in the germination media. This aim research determined the effect of adding various concentrations of growth regulator on the three types of oil palm fruit based on the thickness of the shell. This research carried out at the Tissue Culture Laboratory of PT. Sampoerna Ago Tbk. from September 2018 to January 2019. The growth regulator substances used in this study were NAA, BAP and

GA3. The composition of the growth regulators used were H1 = 0.05 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA3, H2 = 0.05 mg/L NAA + 0.3 mg/L BAP + 0.1 mg/L GA3, H3 = 0.05 mg/L NAA + 0.5 mg/L BAP + 0.1 mg/L GA3 and H4 = No growth regulator. While the types of oil palm fruit used were C1 = Dura, C2 = Tenera and C3 = Pisifera. This research used a factorial complete randomized design with three replications. The results showed that in general the use of growth regulator substances H1 = 0.05 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA3 and the type of dura fruit showed positive results based on viability, scoring, survival and height of plantlets when compared with other treatments.

Keywords: oil palm, in vitro, dura, pisifera, tenera

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the commodities with high economic value in Indonesia that brings foreign exchange to the country. Based on the BPS report in January 2018, the total national exports in 2017 were valued at USD 168.7 billion, consisting of oil and gas exports at USD 15.3 billion and non-oil and gas exports (including oil exports) at USD 152.9 billion. Further, for that total exports in 2017, oil palm exports contributed USD 23 billion (Gapki.id, 2017). To optimally increase the productivity of oil palm product, plantation management through the implementation of best management practices (BMP) are necessary. These cover applying the best technical culture consistently.

That follows the characteristics of the local pedoclimatic and plant conditions and providing everything that the plant needs to achieve its production potential (Imuh, 2017). Moreover, other supporting factors include the use of superior seeds, crop maintenance and harvest technology (Pahan, 2015). As shown on, the oil palm does not produce axillary shoots, making vegetative propagation impossible. Around 98% of oil palm planting material therefore consists of hybrid seeds (Kushairi et al., 2010). Because the oil palm only has one meristem, it must be propagated in vitro by indirect somatic embryogenesis SE (Weckx et al., 2019). Meanwhile, vegetative propagation of oil palm such as tissue culture is still very limited (Sunarko, 2014).

However, the oil palm nurseries using seeds have several constraints. Oil palm

seeds are difficult to germinate because of their hard shell structure, which causes them to have dormancy properties, therefore if they are planted directly in soil or sand without treatment, the germination rate is only 50% after 3-6 months (Fauzi et al., 2012). Besides, oil palm seeds cannot grow simultaneously and are strongly affected by germination chamber conditions (Julyan & Qadir, 2017). Recalcitrant seeds are seeds that are easily damaged that are not resistant to drying and cannot be stored at low temperatures, so they cannot be stored for long (Yuniarti et al., 2016). Germplasm conservation can be done by collecting germplasm in its natural habitat (in situ conservation) or in locations outside its natural habitat (ex situ conservation) (Sumaryono, 2016), it requires a large investment in land, labor and time (Suranthran et al., 2011).

Palm oil fruit types can be distinguished according to the fruit color and shell thickness. Based on the color of young fruit, palm oil is divided into three types, namely Virescen, Nigrescen, and Albescen (Pandian & Matana, 2016). Moreover, according to the shell thickness, it can be divided into three, namely dura, tenera and pisifera. The dura has a thick and hard shells (Figure 1). Next the pisifera does not have a shell. Also, the tenera is the one that has a medium shell thickness (Table 1) (Table 2) (Allorerung et al., 2010). It makes the type that is widely cultivated is tenera. This type is the results of the crossing of dura x pisifera and generally has a thick endocarp thickness and the kernel is quite large. Its thick mesocarp causes high oil production (Hapsoro & Yusnita, 2016).

Palm fruit has a very hard shell. There are 3 main parts of an oil palm fruit. They are the outer layer (*epi carpium*) or outer skin, the middle layer (*meso carpium*) or fruit flesh containing palm oil and the inner layer (*endo carpium*) or the core that contains core oil. Palm oil seeds also consist of 3 main parts, which are seed coat (*spermodermis*) or shell, umbilical cord (*funiculus*) and seed core (*nucleus seminis*). In this core there is an embryo to be a prospective plant. Embryo culture in vitro is a widely used and effective way of overcoming the dormancy nature of seeds in oil palm (Thawaro & Te-chato, 2010). In vitro conservation offers an alternative storage for plant germplasm that has recalcitrant seeds (cannot be stored) or reproduces vegetatively, and provides sterile plant material for germplasm

exchange and for mass propagation of plants (Sumaryono, 2016). Embryo culture functions to save embryos by cultivating them in vitro on aseptic culture media (Taryono, 2015), this medium replaces the function of endosperm (Syukur *et al.*, 2015).

The success of oil palm embryo culture depends on growth media. It needs to contain essential mineral ions, carbon sources, vitamins and other organic supplements. It includes growth regulators and activated charcoal (Table 3). Moreover, in oil palm embryo culture, growth regulators are needed to optimize plant growth (Suranthran *et al.*, 2011). Therefore, this study aimed to discover the effect of growth regulators in the growth and development of oil palm zygotic embryos in vitro by using varieties of dura, tenera, and pisifera.

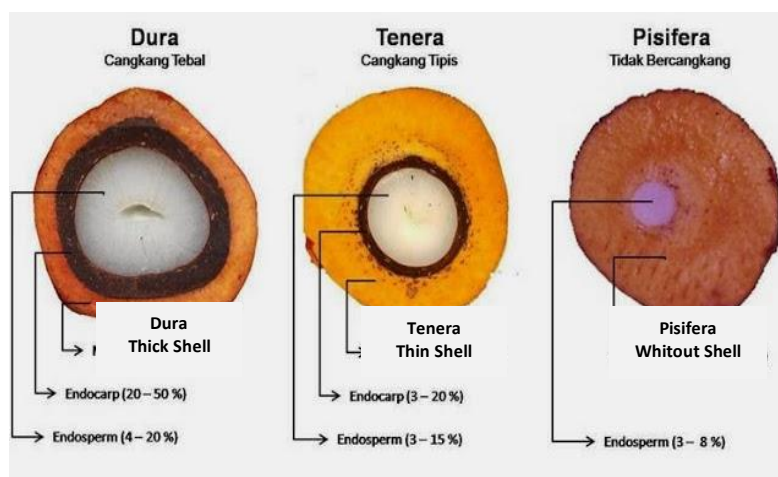


Figure 1. Differences in characteristics of dura, tenera and pisifera fruit types source: (Herbal, 2017)

Table 1. Characteristics of dura, tenera and pisifera types

Characteristics	Dura	Tenera	Pisifera
Shell thickness	Thick	Thin	Without shell
Mesocarp portion of fruit	Low	High	High
Kernel	Big	Moderate	Small
Extracted oil levels	Low	High	High

Source:(Allorerung *et al.*, 2010)

Table 2. Characteristics of types nigescens, virescens and albescens

Characteristic	<i>Nigescens</i>	<i>Virescens</i>	<i>Albescens</i>
Fruit color unripe	Purpel (violet) to black	Geen	Pale yellow
Fruit color ripe	Red-yellow	Orangye	Dark yellow

Source: (Setyamidjaja, 2006)

Table 3. Some arecaceae plants that have been successfully cultivated in vitro by using media types and plants growth regulatory (PG)

Species	Explant	Media	PG	Source
<i>Elaeis guineensis</i>	Embrio	MS	NAA + BAP + GA3	(Suranthran <i>et al.</i> , 2011)
<i>laeis guineensis</i>	Daun	MS	2.4-D + NAA	(Reflini, 2017)
<i>Phoenix dactylifera</i>	Embrio	MS	2.4-D + BA + 2ip	(Bekheet <i>et al.</i> , 2008)
<i>Phoenix dactylifera</i>	Daun	MS	2.4-D	(Kurup <i>et al.</i> , 2014)
<i>Cocos nucifera</i>	Embrio	MS	2.4-D dan Air Kelapa	(Muhammed <i>et al.</i> , 2013)

MATERIALS AND METHODS

Research Implementation

This research was conducted in the tissue culture laboratory, PT. Binasawit Makmur, Km. 10 Palembang from September 2018 to January 2019.

The planting material was in the form of oil palm seeds. They were taken from the research garden of PT. Binasawit Makmur, Surya Adi, Mesuji. Then, they sorted according to their types (Dura, Tenera and Pisifera). After that, the seed shells were broken to extract the kernel. The extracted kernel was stored in a container for further processing. The kernel was sterilized using detergent, bactericide, fungicide, chlorox, and alcohol. The sterilization process was carried out in Laminar Air Flow (LAF).

The basal medium used in this study was (Murashige and Skoog, 1926) in which the addition of 30 g/L sucrose, activated charcoal 2 g/L, and pH 5.8. The dissolved medium was poured into a test tube container with a volume of 15 ml per test tube then sterilized using an autoclave at 121⁰C for 15 minutes and a pressure of 1.5 bar.

The initiation process was carried out in Laminar Air Flow. Prior to the initiation, the kernel was first split for embryo extraction. Then, the successfully extracted embryos were initialized into the medium that had been adapted to the type of treatment. The subculture process was conducted after the second month to induce rooting for one month.

The initiated embryos in the test tube were placed on a culture rack in a dark room (without light) at 26 ± 1⁰C for 2 weeks. After 2 weeks, the test tube containing the embryo was transferred to a

bright room with a long exposure time of 16 hours/day. The irradiation used a 40 watt TL lamp with a room temperature of 23 ± 1⁰C.

Before the acclimatization process, the plantlets were soaked and rinsed with water to clean up the remnants of the medium that were still attached to the roots or stems. They also were soaked first in a fungicide solution (dithane) to avoid fungal attack. The cups/polybags were prepared and filled with top soil, sand, and manure with a ratio of 1:1:1. The enclosure was carried out using a single cup.

Further, the conducted observation parameters were the seed viability, explant growth scoring, plantlet height, plantlet length, plantlet diameter, root length, and mean survival rate of plantlets in acclimatization. Then, the lengthening and rooting aimed to stimulate and enhance the growth of shoots and roots before the acclimation process was carried out. The acclimatization itself was the last stage in tissue culture aiming to move plants (plantlets) from the lab (in vitro) to (ex vitro)/field. Acclimatization was carried out with special techniques so that plants could adapt well to the outside environment (Kumar & Reddy, 2014). The viability was observed based on the visuals of the plantlets. It was measured every two weeks for 8 weeks. The formula for calculating the viability is as follows:

$$V = \frac{N}{T} \times 100\%$$

Notes.

V : Viability

N : Total explants survival

T : Total explants observed

Data Analysis

The method used in this research was a Completely Randomized Design (CRD) Factorial consisting of 2 factors. The first factor was the effect of using growth regulators and activated charcoal, and the second factor was the using of 3 types of oil palm fruit based on the shell thickness. Each treatment combination consisted of 3 replications. The experimental unit was 40 test tubes, each containing one embryo.

First factor:

H1 : 0.05 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA

H2 : 0.05 mg/L NAA + 0.3 mg/L BAP + 0.1 mg/L GA

H3 : 0.05 mg/L NAA + 0.5 mg/L BAP + 0.1 mg/L GA

H4 : Without Growth Regulatory Substances (control)

Second Factor:

C1 : Dura Type

C2 : Tenera Type

C3 : Pisifera Type

The data obtained from the observations were analyzed using Analysis of Variance (ANOVA) to see the effect of the treatment if the results showed a real difference. Then it would be tested further with Duncan Multiple Range Test (DMRT) level of 5%.

RESULTS AND DISCUSSION

The observation results of the embryo growth in the treatments used in this study showed that in general, H₁C₁ and H₂C₁ treatments were the best treatments with the highest percentage of viability, scoring and survival rate (Table 4). The treatment with the lowest percentage of viability, scoring and survival rate was H₄C₃.

The result showed that the H₁ (98.33% and 80.00%) was the best medium according to its highest percentage of viability and scoring compared to other media H₂ (97.50% and 79.17%), H₃ (87.50% and 61.67%), and H₄ (90.00% and 65.00%). Meanwhile, the media which had

the lowest percentages of viability and scoring were H₃ (87.50% and 61.67%) and H₄ (90.00% and 65.00%).

The use of the best fruit type with the highest percentage of viability and scoring was C₁ (98.33% and 80.00%), the dura type, either on H₁ (98.33% and 80.00%), H₂ (97.50% and 79.17%), H₃ (87.50% and 61.67%) or H₄ (90.00% and 65.00%) media. The type of fruit with the lowest percentage of viability and scoring was C₃ (69.17% and 45.83%), either on H₁ (69.17% and 45.83%), H₂ (74.17% and 40.00%), H₃ (65.00% and 30.00%) or H₄ (50.83% and 25.83%).

Zygotic embryos were observed and classified according to scores. The score given on explant growth in this study is 0-5:

- Score 0 is characterized by explants that do not experience growth (no increase in embryonic volume)
- Score 1 is characterized as increasing the length of the embryo but not experiencing swelling
- Score 2 is characterized by increasing length and swelling in the embryo
- Score 3 is characterized only by the appearance of roots or only appears to be budding.
- Score 4 is characterized by the appearance of shoots and leaves but no roots appear
- Score 5 is characterized by the emergence of shoots and leaves and roots, hereinafter referred to as plantlets. This score of 5 will later be processed to the next stage (acclimatization).

The percentage of viability per two weeks did not show a significant increase. In the week second and fourth there was an increase in viability, although the increase was not significant. Whereas in the week sixth and eighth, almost every treatment did not have the viability percentage increment. The grown plantlets were sorted according to make them able to be processed to the next stage, namely acclimatization.

Table 4. Percentage of viability, scoring and survival rate

Media	Viability (%)			Scoring 5 (%)			Survival Rate (%)		
	C ₁	C ₂	C ₃	C ₁	C ₂	C ₃	C ₁	C ₂	C ₃
H ₁	98,33	94,17	69,17	80,00	62,50	45,83	75,83	58,33	29,17
H ₂	97,50	90,83	74,17	79,17	65,83	40,00	71,67	63,33	34,17
H ₃	87,50	85,00	65,00	61,67	57,50	30,00	54,17	54,17	22,50
H ₄	90,00	87,50	50,83	65,00	46,67	25,83	59,17	35,00	20,00

Note: C1 = Dura, C2 = Tenera and C3 = Pisifera, H1: 0.05 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA, H2: 0.05 mg/L NAA + 0.3 mg/L BAP + 0.1 mg/L GA, H3: 0.05 mg/L + 0.2 NAA + 0.5 mg/L BAP + 0.1 mg/L GA and H4: Without Growth Regulatory Substances (control)

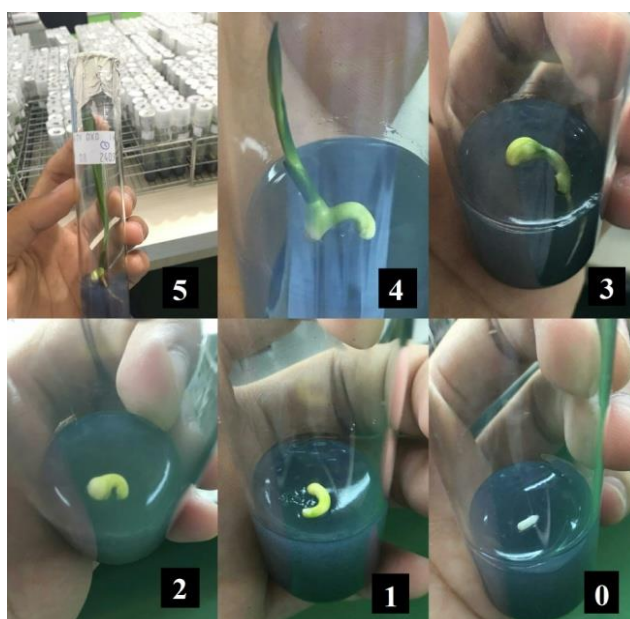


Figure 2. Embryo growth rate scoring

Based on the data, plantlets reaching a score of 5 or were ready to be acclimatized with the total number of 792 or 55% (Figure 2). The best treatment with the highest score of 5 was H1C1 and H2C1 with the percentage score of 5 each 80% and 79.17% respectively. The treatment with the lowest score of 5 was found in H4C3 with the percentage of only 25.83%. Acclimatization data showed that H1C1 and H2C1 treatments had the highest survival rate values compared to the other treatments, that are 75.83% and 71.67%, respectively. While the H3C3 and H4C3 treatments had the lowest survival rate values of 22.50% and 20.00% respectively.

Plantlet Height

The study showed that H1C1 (5.31 cm) and H4C1 (5.02 cm) treatments had the highest average plantlet height compared to the other treatments. Meanwhile, the H₃C₃ (2.48 cm) treatment had the lowest one. The

plantlet height in almost all treatments H1C1 (5.31 cm), H2C1 (5.03 cm), H4C1 (5.02 cm), H3C1 (4.67 cm), H3C2 (4.39 cm), H2C2 (4.36 cm), H1C2 (4.02 cm), H4C2 (3.20 cm), H2C3 (3.14 cm), H4C3 (2.91 cm), H1C3 (2.91 cm) and H3C3 (2.48 cm) had a steady increase on the average, either in the 5th, 7th, or 9th week. Based on the ANOVA, the use of factor C (fruit type) and interaction of H (media) x C (fruit type) significantly influenced the plantlet height. On the other hand, the H factor (media) did not significantly affect the plantlet height (Table 5). Therefore, to find out which treatments on factors C and HxC significantly affected, further testing with DMRT was 5% was conducted.

Based on the further DMRT test results above, it showed that the treatments between C₁, C₂ and C₃ were significantly different from each other. The C₁ treatment had the highest average and the C₃ treatment had the lowest average C1 (5.01

cm), C2 (3.99 cm) and C3 (2.86 cm) (Table 6). Based on the DMRT further test results above, H1C1 (5.31 cm) treatment was not significantly different from the treatments of H2C2 (4.36 cm), H4C1 (5.02 cm), and H3C1 (4.67 cm) but significantly different from the treatments of H3C2 (4.39 cm), H2C2 (4.36 cm), H1C2 (4.02 cm), H4C2 (3.20 cm), H2C3 (3.14 cm), H4C3 (2.91 cm), H1C3 (2.91 cm) and H3C3 (2.48 cm). The H2C1 (5.03 cm) treatment was not significantly different from the treatments of H4C1 (5.02 cm), H3C1 (4.67 cm), H3C2 (4.39 cm) and H2C2 (4.36 cm) but was significantly different from the treatments of H1C2 (4.02 cm), H4C2 (3.20 cm), H2C3 (3.14 cm), H4C3 (2.91 cm), H1C3 (2.91 cm) and H3C3 (2.48 cm).

The H₃C₁ (4.67 cm) treatment was not significantly different from the treatments of H3C2 (4.39 cm), H2C2 (4.36 cm) and H1C2 (4.02 cm) but was significantly different from the treatments of H4C2 (3.20 cm), H2C3 (3.14 cm), H4C3 (2.91 cm), H1C3 (2.91 cm) and H3C3 (2.48 cm). The treatments of each H4C2 (3.20 cm), H2C3 (3.14 cm), H4C3 (2.91 cm), H1C3 (2.91 cm) and H3C3 (2.48 cm) were not significantly different (Table 7).

Root Length

The treatment with the highest average root length was H₄C₂ of 6.189 cm. While

the treatment with the lowest average root length was H1C3 of 0.4 cm. Based on the ANOVA test results, the use of factor C has a very significant effect on root length (Table 8). However, the use of the H factor and HxC interaction did not significantly affect the root length C2 (5.32 cm), C1 (2.82 cm) and C3 (0.59 cm). Therefore, to find out which treatments on factor C had a significant effect, a further DMRT test was performed. Based on the results of further tests above, each factor was significantly different from one another. The factor that had the highest average root length was the C₂ 5.32 cm (Dura) factor. And the factor that had the lowest average root length was the C₃ 0.59 cm (Pisifera) factor.

Stem Diameter

The treatment that had the highest average stem diameter was in the H₁C₂ and H₄C₁ treatments which were 2.8 mm. Whereas the treatment with the lowest average stem diameter was in the H₄ treatment. It was only 2.1 mm. Then based on the ANOVA test results, the treatment did not significantly affect the diameter of the stem, both on the use of the H factor (0.812 cm), C factor (0.350 cm) and HxC interaction (1.446 cm). Therefore, no further tests were carried out on stem diameters.

Table 5. F value calculate for plantlet height, root length and stem diameter

Parameter	Factor H	Factor C	Interaction HxC	CV (%)
Plantlet height	2,495 ^{sn}	85,167 ^{**}	2,912 [*]	9,86
Root Length	1,820 ^{sn}	146,780 ^{**}	2,054 ^{sn}	20,91
Stem Diameter	0,812 ^{ns}	0,350 ^{ns}	1,446 ^{ns}	15,39

Note: ** = significantly different at 1%, * = significantly different at 5%, ns = not significantly different in the DMRT test of 5% level. Factor H = effect of using growth regulators and activated charcoal Factor C= types of oil palm fruit

Table 6. Plantlet height DMRT test results on factor C

Factor C	Height (cm)	DMRT
C ₁	5.01	4,29 _a
C ₂	3.99	3,31 _b
C ₃	2.86	_c

Note: The numbers followed by the same letter mean that they are not significantly different in the DMRT test of 5% level. Factor C = types of oil palm fruit

Table 7. Plantlet high DMRT test results on HxC interactions

Treatment	Average (cm)	DMRT
H1C1	5,31	4,52 _A
H2C1	5,03	4,24 _{Ab}
H4C1	5,02	4,23 _{Ab}
H3C1	4,67	3,88 _{abc}
H3C2	4,39	3,61 _{Bc}
H2C2	4,36	3,59 _{Bc}
H1C2	4,02	3,25 _C
H4C2	3,20	2,45 _D
H2C3	3,14	2,40 _D
H4C3	2,91	2,20 _D
H1C3	2,91	2,23 _D
H3C3	2,48	_D

Note: The numbers followed by the same letter mean that they are not significantly different in the DMRT test of 5% level

Table 8. Results of DMRT root length test on factor C

Factor C	Root Length (cm)	DMRT
C ₂	5,32	4,13 _a
C ₁	2,82	1,68 _b
C ₃	0,59	_c

Note: The numbers followed by the same letter mean that they are not significantly different in the DMRT test of 5% level. Factor C = types of oil palm fruit

DISCUSSION

The implementation of oil palm tissue culture in this study had a fairly high rate. The number of embryos cultured in this study was 1,440 with a success of 792 plantlets measured by the number of acclimatized explants (score 5) for each variety. Meanwhile, if the success percentage were separated among the three varieties, they could reach 80.00% for the dura variety, 65.83% for the tenera variety and 45.83% for the pisifera variety. An even higher number was found in viability, reaching 98.33% for the dura variety, 94.17% for the tenera variety and 74.17% for the pisifera variety. The viability itself was measured by looking at the growth of embryo germination. Embryos that were considered viable were embryos showing germinants growth. Consequently, many of the embryos that showed bud growth. Therefore, there were many of the embryos that had emerged shoots but the growth was not optimal so it did not result a perfect plantlet.

The level of contamination was very small. There were only 11 out, of the 1440 explants initiated that were contaminated or

equal, to 0.77%. The source of contamination came from fungi and bacteria. The small level of contamination was due to the implementation of tissue culture following the standards set by PT. Binasawit Makmur. Mastuti, (2012) stated that the contamination that occurs is caused by microorganisms both originating from outside (exogenous) or from explants (endogenous) due to the procedural errors when performing tissue culture.

The use of growth regulators with a concentration of 0.1 mg/L cytokines was the best medium at plantlet height. However, a higher concentration of cytokinins reduced the average height of plantlets the same as without the use of growth regulators. During the stage of plant multiplication in vitro, the auxins are used in low concentrations, and accompanied by high concentrations of cytokinins which stimulate shoot proliferation (Su *et al.*, 2011). Therefore, the optimal growth regulator for use in oil palm tissue culture was 0.05 mg/L NAA + 0.1 mg L BAP + 0.1 mg/L GA3. This study was in line with the one conducted by (Suranthran *et al.*, 2011) that in his study the use of 0.1 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA3 produced

the best average plantlet height compared to other treatments with lower concentrations of growth regulating substances.

The plantlet height developed a steady increment every two weeks from the third week to the ninth week. The increase of plantlet height ranged from approximately 1 to 2 cm every two weeks. In the ninth week, the highest plantlet was found in the H1C1 treatment. It was 5.312 cm. This figure was arguably low for the plantlet age of 9 weeks. The reason was that in the study (Suranthran *et al.*, 2011) the plantlet height could reach 9.43 cm in the fourth week. The low plantlet height was due to not having sub-culture so that the availability of nutrients and growth regulators was always reduced.

Their most important effects in the *in vitro* cultures are enhancement of root and shoot production, stimulation of callus cell divisions, differentiation of conductive tissues, cell elongation, and inducing apical domination (Singh *et al.*, 2016). The presence of growth regulators in tissue culture is very important. The growth regulators in tissue culture function to control organogenesis and morphogenesis in the formation and development of shoots and roots and callus formation. The growth regulators which are often used in tissue culture are auxin and cytokinin groups (Lestari, 2011). The use of auxins and cytokines in tissue culture usually depends on the direction of growth. If shoots growing is wanted, the ratio of cytokinins is higher than auxin, and vice versa (Mastuti, 2017).

Auxin treatments resulted in plantlets with greater shoots than plantlets under cytokinins only, probably from the higher *in vitro* rooting that contributed to the elongation of plants (Victório *et al.*, 2012). The auxin used in this study did not vary or was the same among the treatments, so it was natural that the use of growth regulators here had no significant effect. However, in the treatment without using growth regulators (control), the root length data also still had no significant effect. Those results probably caused by the use of auxin

concentrations which were too low so that the difference in treatment could not be seen, whether the treatments using growth regulators or not. These were in line with the research conducted by (Suranthran *et al.*, 2011) which showed that using auxin 0.1 mg/L was significantly different from the one using auxin of 0.05 mg/L and without using auxin. While the use of auxin of 0.05 mg/L and without using auxin each was not significantly different.

CONCLUSION

Referring to the purpose of the study the results of this study where the use of H1 media = 0.05 mg/L NAA + 0.1 mg/L BAP + 0.1 mg/L GA3 + 2000 mg/L activated charcoal can increase the growth of oil palm embryos *in vitro*. In addition to the type of fruit C1 = dura has better growth than other types of fruit, this is due to an increase in cytokines in this study have not been able to increase the growth of oil palm embryos from the types of dura, tenera and pisifera; and In general, the best treatment in this study was H1C1.

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