Organogenesis of palm oil from apical bud explants

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Abstract. The purpose of this research is to find out explants apical bud from oil palm of different position which are apex, middle, and basal segment, were cultured in Y3 medium supplemented with 2,4-D in the presence of activated charcoal as many 3 g/L for induction organogenesis from callus inisiation. This research is conducted at Tissue Culture Laboratory, Mathematic and Natural Science Faculty, North Sumatera University from November 2013 to June 2014. In this research, experiment for organogenesis by growth regulator substance 2,4-D at concentration of 100 mg/L, 115 mg/L, 120 mg/L, 135 mg/L, and 140 mg/L. This research was designed by completely randomized design two factorials with 5 attempt. The production of organ was affected by 2,4-D concentration and explants position; 2,4-D 100 mg/L was the most effective (66%) in inducing organogenesis from the basal segment.

1. Introduction

Oil palm (Elaeis guineensis Jacq) is one kind of plants that has long been cultivated as plantation and a plant that originated from Africa and South America. In South America, Africa, the South Pacific and Southeast Asia, palm oil is a type of commercial crops. Indonesia is one of the Southeast Asian countries that make palm oil as a commercial crop, especially as a plant that could increase foreign exchange [1].

Oil palm is a perennial plant that comes from Africa with a regeneration period of about 19 years and in the process of marriage takes very slow. Based on the formed fruit shell thickness, consisting of three varieties; dura, psifera, and tenera. Tenera is a type of super palm seeds, for it has thin fruit shells, but females flower is fertile. Tenera naturally produce more palm oil than the Dura and Psifera varieties. The fruit percentage can reach 90% and the oil content can reach 28% per stem [2].

Along with the increasing consumption of vegetable oils in the world and particularly in Indonesia, it is not possible preservation of palm oil is still being done conventionally. Therefore, an alternative that can be taken for solving such problems is by tissue culture techniques that can produces similar seeds and with tissue culture techniques can be obtained.
superior planting material in a short time [3]. Organogenesis is the process of development or adventitious roots from within callus cells. Organogenesis process can occur directly, namely the initiation of explant and indirectly through the formed callus initiation during embryogenesis.

Based on the growth regulator for callus formation, so in the planting media should be added auxin and cytokinin. These two substance interactions effect the growth, morphogenesis within the cell culture, tissue culture and organ. The concentration of the growth regulator is often controlling the shape and the amount of growth in a culture, both callus growth and organogenesis. As a general guide auxin or cytokinin or both are added into the culture to obtain a growth response [4]. The process of organ formation in vitro is strongly influenced by the origin of explant source and composition of the used growing media. Media composition adapted to the plant type [5].

The culture media composition and the used growth regulators concentrations during organogenesis is an important factor of embryogenesis multiplication that will produce organ in the plantlets forming. Auxin plays an important role in the growth and development of plants. Generally auxin plays a role in cell division, elongation and the cell differentiation, as well as signals between cells, tissues, and organs of plants. The existence of auxin in the medium will affect the process of initiation and root growth. The appropriate auxin combination and concentration can increase the percentage of callus and percentage of plantlets in vitro root [6].

2. Materials and methods

The used chemicals is the media arranger components Y3 (Eeuwens), growth regulator 2.4-Diclorophenoxyacetic (2,4-D), HCl 0.1 N and 0.1 N NaOH to obtain a pH of 6.0. Sterilization using ascorbic acid, fungicides, sodium hypochlorite, tween 80, betadine, solution of sodium hypochlorite 1% and 5%, and detergents. The equipment used is autoclave laminar air flow, and glassware. The embryogenic which has been sub-cultured from explants of apical bud crude origin with different zones is the source of explants in the organogenesis on this study. Experimental method used is the method completely randomized design (CRD) (Rancangan Acak Lengkap (RAL) factorial with 2 factors, namely: I. taken explants segment factor (regional apex, middle region, the regional basal), II. The differences of concentration factors of growing regulator 2.4 - Diclorophenoxyacetic (100 mg / L, 115 mg / L, 120 mg / L, 135 mg / L, 140 mg / L).

Table 1. Position of Explant and concentration of plant growth regulator

| Concentration  | Explants Position |
|----------------|-------------------|
| 2,4-D          | A | M | B |
| 100 mg/L       | P₁A | P₁M | P₁B |
| 115 mg/L       | P₂A | P₂M | P₂B |
| 120 mg/L       | P₃A | P₃M | P₃B |
| 135 mg/L       | P₄A | P₄M | P₄B |
| 140 mg/L       | P₅A | P₅M | P₅B |
3. Results and discussion

3.1 The Time of Organ Formation

Average of organ formation in explant segments position with some degree of concentration through callus initiation diversify growth rate variation at the beginning of the formation of organs in the growing media and the average initial formation of organs occurs in the basal segment position with concentration 2.4 D of 100 mg / L (Figure 1). The development of organ formation through the initiation of callus derived from the explant segments position of the different apical bud (position of apex segments, segments median position, and the position of the basal segment) has the development of uneven organ formation. At the position of the basal segments forming organs faster than the median segment position and the position of the apex segment., [7] stated that the formation of the organs affected by the division of embryonic callus cells to form embryonic somatic cells are ready to differentiate into organs. Growth regulators substance exogenous and endogenous hormones play a role in activating the cell metabolism. [8] stated that the ability of cells to divide associated with genotypes and origin of explants used. The used explants mother source contains different endogenous hormone.

![Figure 1](image)

**Figure 1.** The average of forming organ on explants position with some concentration degrees 2,4 D

3.2 Percentage Explant Forming Organs (%)

The percentage of callus initiation of apical bud explants with position apex segments, median, and basal form organs is at 28% as many as 21 explants of 75 explants. The average of forming organ occurred in 13th after initiation of callus is showed at Figure 2. Average of forming organ through callus initiation at a position of higher basal segments is showed at Table 2. From 21 explants forming organs, the explants basal position was (40%) and 2,4-D concentration was 2,4-D of 100 mg / L (66%) is the position of explant and concentration of 2,4-D that form the highest organ.
Table 2. The average of forming culture callus organ of explant *apical bud* of palm oil at several concentration levels of 2,4-D and the position of the different segments of explants.

| Concentration of 2,4-D | Explants Amount | Explants Position | Average |
|-----------------------|-----------------|-------------------|---------|
|                       |                 | A     | M     | B     |       |
| P₁                    | 2               | 3     | 5     |       | 3,33<sup>c</sup> |
| P₂                    | 1               | 3     | 2     |       | 2,00<sup>bc</sup> |
| P₃                    | 0               | 2     | 3     |       | 1,67<sup>b</sup> |
| P₄                    | 0               | 0     | 0     |       | 0,00<sup>a</sup> |
| P₅                    | 0               | 0     | 0     |       | 0,00<sup>a</sup> |
| Average               | 0,60<sup>a</sup> | 1,60<sup>abc</sup> | 2,00<sup>bc</sup> |
| F (A: 2,4-D)          |                 | 9,100<sup>*</sup> |
| F (B: explants position) |                 | 3,900<sup>*</sup> |
| F (A x B)             |                 | 1,150<sup>ts</sup> |

Description: Values followed by the same letters in the same column are not significantly different at the Duncan test (p <0.05). * P <0.05; ts: not significant.

In line with the results of research of [9], the best palm oil generation is produced in the growing medium with the lowest concentration of growth regulators with 2,4-D concentration of 100 mg / L. Exogenous hormones on growth media works synergistically with endogenous hormone produced by explants. Exogenous hormone 2,4-D was added to the growing medium in which a small amount of concentration is more effective in inducing the formation of callus initiation organ through the growing medium. The basal segment position is an composed explant position on embryogenic meristematic tissue also more effective concerning forming the organ through callus initiation on the growing medium. The concentration of 2,4-D corresponding to the growing media with the position of the segment that contains many cells of meristematic increase the organ growing speed.

3.3 The Wet Weight Organ

Wet weight pupus organ weight that is formed through the initiation of callus from different explants position segments showed varies responses. There is an interaction between plant growing regulators substance of 2,4-D to the position of explants that provide significant influence on organ wet weight pupus with the level of interaction the best treatment is found at the level of interaction between basal segment position with 2,4-D concentration level of 100 mg / L (Table 3).
Table 3. The average of wet weight of forming pupus organ of callus culture from explant apical bud of palm oil at several concentration levels of 2,4-D and the position of the different segments of explants.

| Concentration 2,4-D | Dry weight (g) | Average |
|---------------------|----------------|---------|
|                     | A              | M       | B       |
| P_1                 | 0.20^{a}       | 0.41^{b} | 1.16^{c} | 0.59^{c} |
| P_2                 | 0.06^{a}       | 0.34^{b} | 0.32^{c} | 0.24^{b} |
| P_3                 | 0.00^{a}       | 0.15^{b} | 0.38^{c} | 0.18^{b} |
| P_4                 | 0.00^{a}       | 0.00^{a} | 0.00^{a} | 0.00^{a} |
| P_5                 | 0.00^{a}       | 0.00^{a} | 0.00^{a} | 0.00^{a} |
| Average             | 0.05^{a}       | 0.18^{b} | 0.37^{c} |         |

F (A: 2,4-D) = 18.796^{*}
F (B: explants position) = 13.877^{*}
F (A x B) = 4.976^{*}

Description: the values followed by the same letters in the same column are not significantly different at the Duncan test (p < 0.05); * P < 0.05. Abc notation: interaction concentrations of the position; xyz notation: interaction position to concentration.

According to [2] through the culture of palm explants of leaf organ explants expressed the need organ initiation of somatic embryogenesis in the development of tissue explants very largely determined by the concentration of 2,4-D that is appropriate to the growing medium and the number of high cells of meristematic explants.

Elongation and cell division are affected by plant growing regulators substance and the amount of carbohydrates in culture. According to [3] auxin in cell division plays a role in increasing the turgor cell pressure and high sucrose content in the growing media may work synergistically with endogenous cytokinin hormone to enhance the development of embryogenic to generate buds. According to [7] exogenous auxin 2,4-D increases the cycle of cell division in callus-meristematic cell micro embryogenetic.

3.4 The Dry Weight Organ

Concentration 2,4-D of 100 mg / L and the position of the basal segment showed the pupus highest dry weight (Table 4). The high organic biomass increases the rate of cell metabolism. Cell metabolic rate is directly proportional to the rate of growth and development of somatic embryo forming organs. The rate of cell metabolism is influenced by the presence of growing regulators. Appropriate growing regulator will increase the rate of cell metabolism in the cells of the callus. Somatic Callus derived from embryogenic callus is meristematic cells have a high metabolic rate. Meristematic cells that already have prokambium, protoderma continue to actively divide to form zones cambium, epidermis and different to form fat reserves in somatic embryos. Somatic embryogenesis is a process by which somatic cells to form organs evolved through the stages of formation of zygotic embryos [8].

According [4] embryozygotic cells that mature faster and are meristematic produce plantlets. Plantlets formed from callus cell division activity embryozygotic meristematic cells contain cytoplasm rich in organic compounds. [7] found that the addition of exogenous hormone auxin 2,4-D into the growing medium can increase cell division cycle meristematic
region during the transition period somatic embryogenic. Embryogenic somatic cell division activity continuously will increase the accumulation of the organic content of the cytoplasm of cells such as proteins and polikasarida (starch). Starch and protein accumulation is an indicator of good development and good growing embryogenesis.

**Table 4.** The average of dry weight of callus forming the pupus organ callus culture from palm explant *apical bud* on several concentration levels of 2,4-D and the position of the different segments of explants.

| Concentration 2,4-D | Dry weight (g) | Explants position | Average |
|--------------------|----------------|-------------------|---------|
|                    |                | A     | M     | P     |       |
| P1                 | 0,03ay         | 0,06ay | 1,18bx | 0,09c |
| P2                 | 0,01ax         | 0,04ax | 0,04ax | 0,03b |
| P3                 | 0,00ax         | 0,02ax | 0,04ax | 0,02ab|
| P4                 | 0,00ax         | 0,00ax | 0,00ax | 0,00a |
| P5                 | 0,00ax         | 0,00ax | 0,00ax | 0,00a |
| Average            | 0,01a          | 0,02a  | 0,05b  |       |

*Description: the values followed by the same letters in the same column are not significantly different at the Duncan test (p <0.05); * P <0.05. Abc notation: interaction concentrations of the position; xyz notation: interaction position to concentration.*

![Figure 2](image_url). The formed bud organ through callus initiation from *epical bud* explants. (A) age 3 week; (B) age 6,5 week; (C) age 8 week; (D) age 8,5 week; (E) age 9,5 week; (F) age 11,5 week.
4. Conclusion

The production of organ was affected by 2,4-D concentration and explants position; 2,4-D 100 mg/L was the most effective (66%) in inducing organogenesis from the basal segment.

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