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ENDOGENOUS HORMONES FROM THE YOUNG LEAVES OF OIL PALM (*Elaeis guineensis* Jacq.): A RESPONSE TO *In Vitro* CALLUS AND EMBRYOID INDUCTION

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ABSTRACT

Oil palm is a plant that produces vegetable oil. Quality oil palm seedlings with superior characteristics can be produced clonally using tissue culture techniques. The selection of explant sources is very important, and one of the explant sources that can be used is the young oil palm leaves. The ability of callogenesis and embryogenesis remains low, and this is thought to be related to endogenous hormones. This study is aimed to identify the types of endogenous hormones in explants of young clonal oil palm leaves and their relationship to callus and embryoid induction. The leaf midribs of six young oil palms were selected as explant sources. Explants were extracted and analysed for endogenous hormone content using high-performance liquid chromatography. The results of the analysis obtained various types of endogenous hormones in four oil palm clones. Zeatin hormones, ABA and IBA were found in two clones. Nine hormones were found in the other two clones, namely zeatin, kinetin, gibberellic acid, IAA, BAP, ABA, IBA, 2,4-dichlorophenoxyacetic acid, and naphthalene acetic acid with various concentrations. Young oil palm leaves with a more complete content of endogenous hormones have the potential to produce embryogenic callus in greater numbers and can produce embryoids to ensure that they can support the production of plantlets using tissue culture technology.

Keywords: ABA, benzyl amino purine (BAP), embryogenic callus, zeatin, 2,4-D.

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INTRODUCTION

Oil palm is an economically important plant, with almost all plant's parts able to be utilised, most importantly its oil can be processed into various food and non-food products. The oil palm fruit consists of a pulp (mesocarp) and a kernel which contains about 45%-50% (Owoyele and Owolobi, 2014) and 47% of oil (Naher *et al.*, 2013), respectively. Compared with other oil-producing plant commodities such as soybean, sunflower, canola and corn, the oil palm remains as a prime commodity due to its much higher oil productivity per planting area (Naher *et al.*, 2013).

The tissue culture technique has been introduced in oil palm plant-related activities since 1970 to meet the demand for superior seedlings (Ismail *et al.*, 2010; Staritsky, 1970). Oil palm plant has only one growing point (single apical meristem) which makes it difficult to propagate vegetatively in the field, where tissue culture technology offers a solution (Mariani *et al.*, 2014). Oil palm tissue culture requires explants sources that can be derived

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from young leaves, roots, flowers (inflorescence), and embryos (Weckx *et al.*, 2019), each having its advantages and disadvantages. Young leaves are mostly selected as the source of explants for oil palm tissue culture because of their availability in large quantities. One ortet can produce hundreds or even thousands of explant pieces with relatively higher sterility (Setiowati *et al.*, 2013).

The tissue culture technique has been applied to generate quality oil palm seedlings with increased crude palm oil productivity (Weckx *et al.*, 2019). The resulting clonal seedlings possess quality characteristics identical to their parents and can be produced without season constraints (Soh *et al.*, 2011). The *in vitro* clonal propagation of oil palm, however, still faces the problems of poor productivity of callus and somatic embryo, with the callogenesis and embryogenesis rates as low as 14.00%-19.00% and 3.00%-7.00% (Kushairi *et al.*, 2010), 30.00% and 5.00% (Alwee *et al.*, 2010), 10.80% and 3.00% (Yusnita and Hapsoro, 2011), 33.33% and 7.69% (Sanputawong and Te-Chato, 2012), 15.00% and 3.00% (Marbun *et al.*, 2015), 1.39%-30.56% and 3.63% (Wiendi *et al.*, 2015), 13.10%-22.30% and 5.00% (Gomes *et al.*, 2017), as well as 20.48% and 3.00%-5.00% (Karyanti *et al.*, 2019), respectively. The use of broodstock sources with different genotypes is thought to contain different endogenous hormones and affect callus and embryoid induction abilities (Elhiti *et al.*, 2013).

The quality of the callus formed in oil palm tissue culture is another crucial issue since its quality affects the ability to form embryoids (or embryonic calli) which are the precursors of the plantlets to be produced. Callus formation is induced by the interplay of both exogenous and endogenous hormones. The use of quantifiable hormone concentrations can suppress the occurrence of somaclonal variations, especially when young leaf explants are used (Bairu *et al.*, 2011). The administration of measurable synthetic hormones was unable to increase the number and quality of the callus formed, triggering speculation that the problem lay in the unidentified endogenous hormones present in the source explants. These auxin and cytokinin types of endogenous hormones could affect the callus and embryoid formation (Xue *et al.*, 2020). Thus, this study aims to measure the content of endogenous hormones in the young leaves of oil palm plants and to determine their effect on *in vitro* callus and embryoid induction.

MATERIALS AND METHODS

This study was carried out at the Biotechnology Laboratory, National Research and Innovation Agency, Science and Technology Park, South Tangerang City, Banten, Indonesia. The activities consisted of *in vitro* plant tissue culture in the Tissue Culture Laboratory and hormone measurement in the Laboratory of Analytical Chemistry.

Explant Source for Callus Induction

Young leaves were sampled from four oil palm trees (broodstocks) of different genotypes which had been determined beforehand, based on normal morphological data and high production. The selected broodstock was the result of *Dura* Deli × *Pisifera* Nigeria Origin, hereinafter referred to as a clone. Sampling was conducted in 2018 from oil palm plantations located in Kumai, Central Kalimantan. All four oil palm trees used as a source of explants were about 7 years old and denoted as clone-1, clone-2, clone-3, and clone-4 (*Figure 1a*, *b*, *c* and *d*). The shoots of each clone were carefully cut to avoid damaging the growing point and were brought to the laboratory (*Figure 1e*).

The young shoots were cleaned and carefully opened, exposing the midrib layers (*Figure 1f*). The six youngest leaf midribs commonly used in oil palm tissue culture labelled as midrib layer numbers 5, 6, 7, 8, 9 and 10 (*Figure 1g*), were used as the source of explants for callus induction and as sample for analysis of endogenous hormone content (*Figure 1h*). Each midrib was cut into pieces of about 1.5 cm (*Figure 1i*). The young leaf pieces to be used as explant for callus induction were sterilised (*Figure 1j*), and then planted on a callus induction medium (*Figure 1k*).

Explant Source for High-Performance Liquid Chromatography (HPLC) Analysis of Endogenous Hormones

A method similar to the one for preparing the explants for callus induction was used, but with no sterilisation. The explant sample was weighed out of 100 g (*Figure 1j*) and extracted before analysis using HPLC. Each sample was replicated three times.

Endogenous Hormone Analysis

The hormone was analysed using the HPLC method as described by Li *et al.* (2016) with modification. The explant samples were macerated using ethyl acetate (Emsure) and then homogenised using a magnetic stirrer for approximately 24 hr. The macerated samples were filtered (Whatman 45 mm), and the filtrate obtained was centrifuged for 10 min at 3000 rpm. The centrifuged filtrate was separated from the solid and evaporated (Heidolph Laborota 4000 Rotary Evaporator) to dry, to derive endogenous

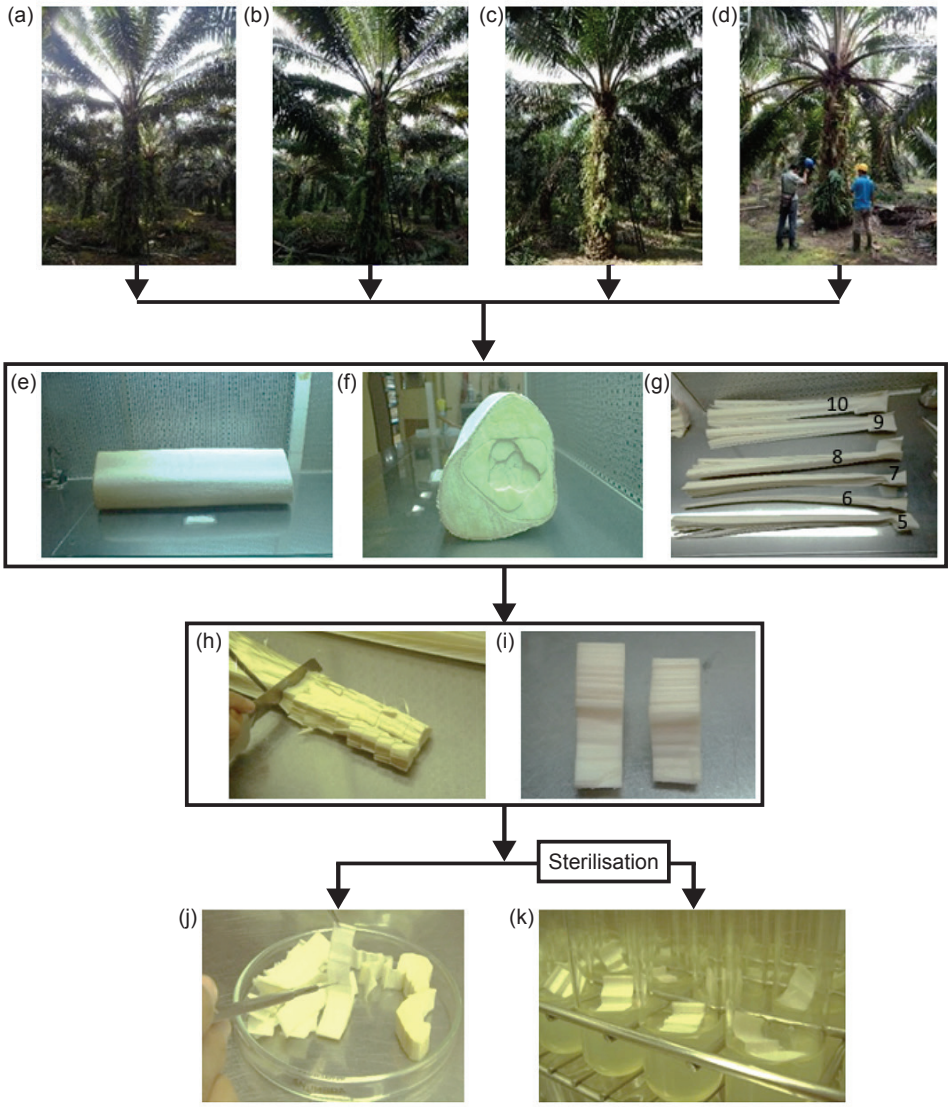

Figure 1. Source of explants for callus induction and endogenous hormone analysis: (a) clone-1, (b) clone-2, (c) clone-3, (d) clone-4, (e) shoots sampled from each clone, (f) cross-sectional area of shoots after cutting, (g) six midribs selected and numbered 5, 6, 7, 8, 9, and 10, (h) each midrib cut into pieces, (i) explant sized to 1.5 cm pieces, (j) leaf explants for endogenous hormone analysis, and (k) sterilised young leaf explants planted in callus induction media.

growth hormone extract which was subsequently dissolved in 5 mL of methanol (Merck). This methanolic solution was then concentrated again using a vacuum concentrator (Sakuma Ec-2000) for about 3 hr to evaporate the methanol. The concentrated extract was then dissolved again in 500 µL methanol and later centrifuged at 1200 rpm for 10 min to separate and remove any remaining precipitate from the hormone extract solution sample, to be assayed using an HPLC (Hitachi L-2130).

HPLC analysis was performed by injecting the extract solution sample (10 µL). The mobile phase used was methanol:formic acid 0.1% pH 3.2 with a ratio of 10:90 and was run isocratically at a flow rate of 1.44 mL min^{-1}. Samples were separated using Phenomenex® Luna C18, 5 m (250 × 4.6 mm) chromatographic column and detected using a 200 nm UV detector.

Callus and Embryoid Induction

Young leaf explants were sterilised by submerging in 0.35% sodium hypochlorite (Sigma) solution for 20 min with occasional shaking, followed by immersion in a 0.02% glucose solution (Sigma). The explants were then grown on callus induction medium (a combination of basic Murashige and Skoog medium (Merck) with 0.002-11.050 mg L^{-1} of 2,4-D (Sigma) and incubated in the dark at 26°C-27°C. Callus would form in the fourth month onwards, and the leaves associated with the growing callus were sampled every month. The callus was isolated and transferred onto an embryo induction medium [basic medium MS with 0.002-11.050 mg L^{-1} of 2,4-D and 0.002-11.050 mg L^{-1} of benzyl amino purine (BAP) (Sigma)]. Embryoids would appear at different times. Observation of the callus and embryo induction was done weekly for

12 months, and the number and emergence time of the embryos were recorded (Rismayanti *et al.*, 2019).

Statistical Analysis

For each clone, only three leaf samples (replicates) were taken due to the limited quantity of young palm leaves. Not all sampled leaves indicated endogenous hormone production on HPLC analysis. Thus, statistical analysis was carried out on the clones identified as containing endogenous hormones. The obtained data were normalised and later analysed using the IBM SPSS program (Version 26). Results were further tested for significance using the Duncan Multiple Range Test at the 5% confidence level.

RESULTS AND DISCUSSION

Endogenous Hormone Content

This study analysed the endogenous hormones in the explants of young palm leaves to determine the effect of hormones on callus and embryoid induction. The high chromatographic peak for all the nine hormone standards (Sigma) were obtained, namely 3-methyl-trans-2-butenylamino purine (zeatin), 6-furfuryl amino purine (kinetin), gibberellic acid (GA_3), indole-3-acetic acid (IAA), BAP, abscisic acid (ABA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene acetic acid (NAA). The results of the HPLC measurement of the palm leaf extract samples were referred to as the standard chromatogram (*Figure 2a*).

HPLC analysis on clone-1 (*Figure 2b*) dan clone-2 (*Figure 2c*) showed that not all hormones were identified except zeatin, ABA, and IBA which were detected. These unidentified hormone types seemed to have very low content and, thus, were not readable. Conversely, the chromatogram of clone-3 (*Figure 2d*) and clone-4 (*Figure 2e*) showed all types of endogenous hormones analysed. These results indicated that the clone genotypes showed variations in the type and concentration of endogenous hormones, and this possibly had an effect on growth and development in the field or *in vitro* tissue culture (Correa *et al.*, 2016).

Plant parts contain different endogenous hormones, which depend on the plant organ function and the season (Vedenicheva *et al.*, 2018). The content of endogenous hormones, ABA and zeatin in *Curcuma alismatifolia* Gagnep. plant increases outside the growing season and is stored in the leaves, rhizomes and roots (Hongpakdee *et al.*, 2010). The endogenous hormone cytokinin was found to play a role in the anthesis of macadamia crosses (Trueman, 2010). In the initiation stage of the pineapple flower, it was found that the endogenous hormone content of zeatin, IAA, and 2-isopentenyl adenine (2ip) was low, whereas that of ABA and GA_3 was high (Sheng-hui *et al.*, 2010). In this study, the endogenous hormones zeatin, ABA, and IBA were found in clone-1, clone-2, clone-3 and clone-4 young palm oil leaves. The concentration of the hormones in each sample analysed was not the same, especially the standard deviations (± sd) in clone-1 and clone-2 which were quite high. Similar results were reported, in which only IAA and ABA were found in the leaf explants of *Pennisetum purpureum* Schum. (Rajasekaran *et al.*, 1987).

The graphs of the zeatin content show high standard deviations (*Figure 3*) because not all the individual samples of the young palm leaves show quantifiable hormone concentration on HPLC analysis. The highest average zeatin content was found in the young leaf explants of clone-1 (97.70 mg L^{-1}) and clone-2 (80.16 mg L^{-1}) (*Figure 3*). Zeatin is found in many plants and a certain period such as after pollination for fruit formation (Vedenicheva *et al.*, 2018). High zeatin content was also found in etiolated plants (Grzyb *et al.*, 2017). Besides zeatin, the highest ABA was found in clone-1 (6.72 mg L^{-1}) and clone-2 (6.83 mg L^{-1}).

The endogenous hormone ABA is found in many plants and plays a role in the induction of dormancy, inhibition of germination, stomata regulation and abiotic stress resistance (Finkelstein, 2013). Certain doses of ABA help plants grow, whereas in high doses it inhibits growth (Finkelstein, 2013). Additionally, ABA is also involved in eliminating viruses (Alazem *et al.*, 2014; Alazem and Lin, 2017). The highest concentration of the endogenous hormone IBA was found in clone-3 (10.53 mg L^{-1}) and clone-4 (5.90 mg L^{-1}). IBA is an auxin group hormone that has not been widely discussed in various endogenous hormone research.

The other endogenous hormones such as kinetin, GA_3, IAA, BAP, 2,4-D and NAA were only found in the young leaf samples of clone-3 and clone-4 (*Figure 4*). Endogenous hormones not detected in this study do not mean it is not present, but it could be due to their low content, below the minimum detectable limit of the HPLC. Clone-3 and clone-4 contained various endogenous hormones. Kinetin, zeatin, and BAP are cytokinin group that plays a role in cell division and plant growth support. Endogenous cytokinin helps the embryogenesis process of young leaf explants (Grzyb *et al.*, 2017).

Generally, the content of 2,4-D was found to be higher than that of kinetin, GA_3, IAA, BAP and NAA (*Figure 4*). The highest 2,4-D content was found in clone-3 (2.53 mg L^{-1}) and clone-4 (2.58 mg L^{-1}) (*Figure 4*); and it was not detected

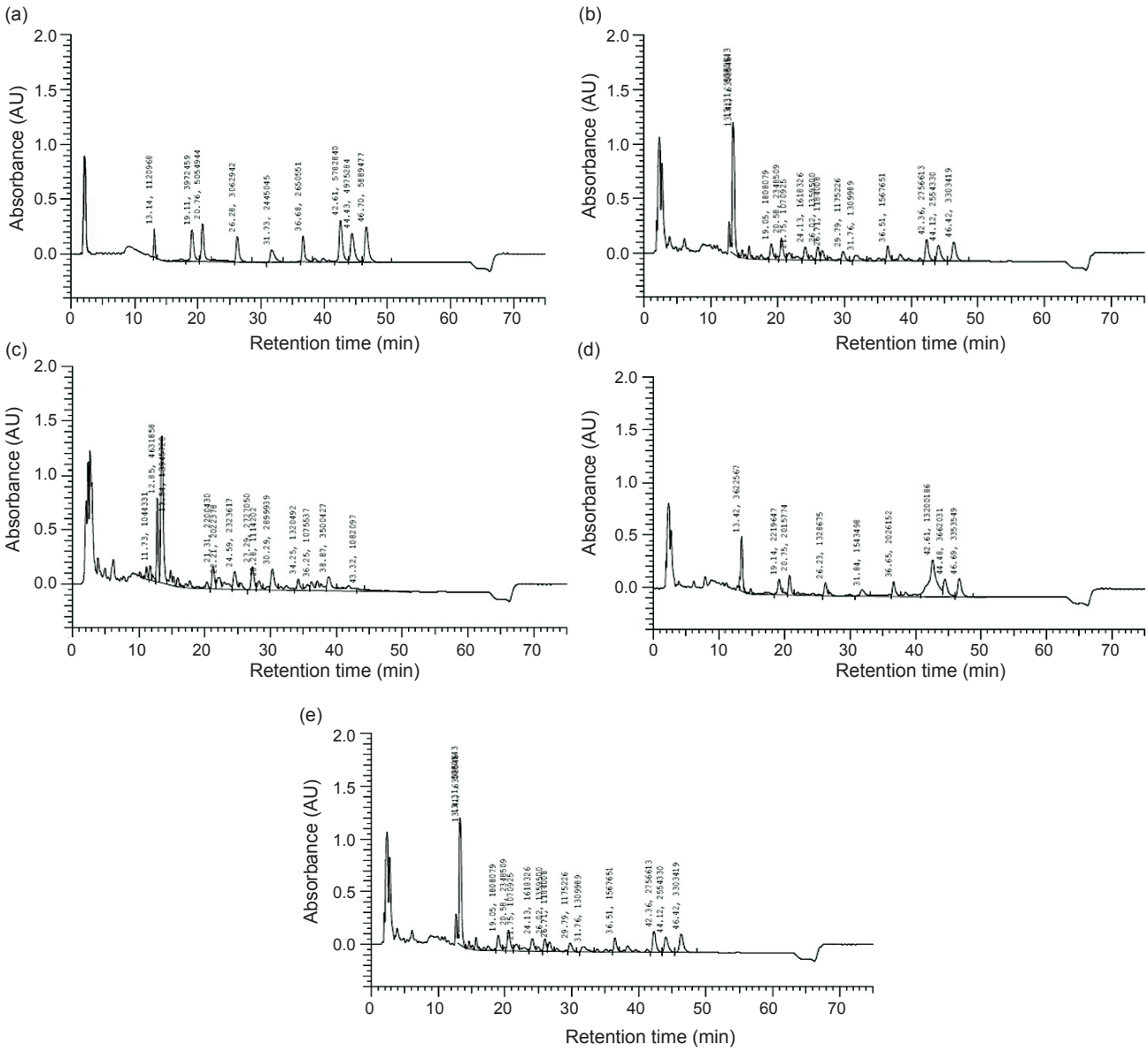

Figure 2. Chromatogram representatives of the endogenous hormone analysis on the oil palm young leaf explants: (a) plant hormone standards, (b) clone-1, (c) clone-2, (d) clone-3, and (e) clone-4.

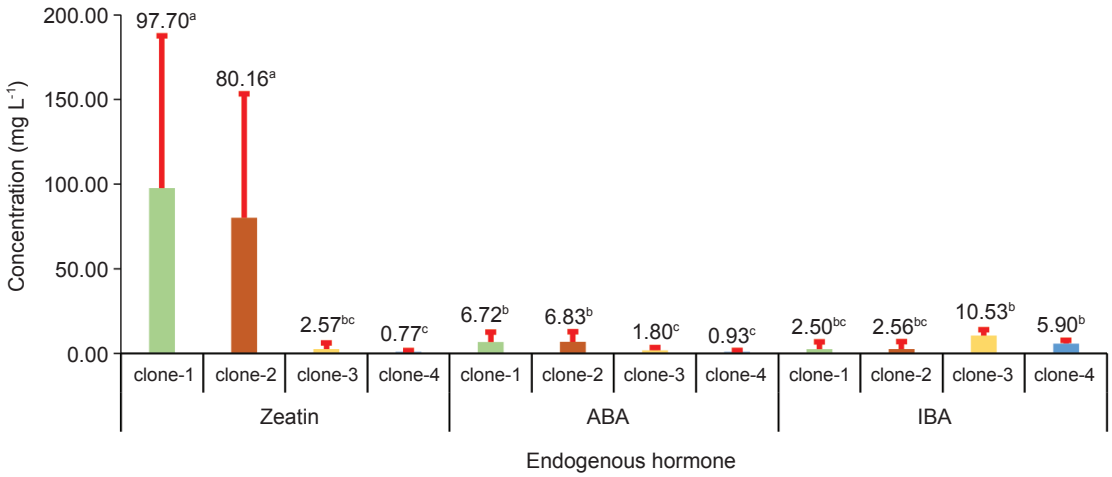

Figure 3. Endogenous hormone content of zeatin, ABA, and IBA in the oil palm young leaves of clone-1, clone-2, clone-3, and clone-4. The graph shows the mean values (± sd) of 3 replicates, followed by the Duncan test with a 0.05 confidence level. Numerical values with different letters indicate significant differences (Zeatin: 3-methyl-trans-2-butenylamino purin, ABA: abscisic acid, and IBA: indole-3-butyric acid).

in clone-1 and clone-2. Kinetin, GA_3, IAA, BAP, and NAA were present in all the clones with concentrations that were not significantly different (p>0.05) (*Figure 4*). Even at low concentrations, endogenous hormones influence plant physiology.

The endogenous hormone IAA or auxin is synthesised in the shoots and increases in concentration in non-merismatic tissues (Ogunyale *et al.*, 2014). IBA is an endogenous hormone of the auxin group which is produced in the shoots, distributed throughout the plant body, and is effective in assisting root induction. BAP belongs to a group of cytokinin that plays a role in cell division to support plant growth. GA_3 is often found in plants and facilitates normal plant growth by accelerating cell division. GA_3 promotes cell elongation, thereby increasing the flowering process and leaf weight as well as triggering seedless fruit production (parthenocarpy).

Effect of Endogenous Hormones on Callus Induction

Explant samples of young oil palm leaves were grown on modified callus induction media, which generally consisted of macronutrients, micronutrients, vitamins, amino acids and measurable growth regulators (Rismayanti *et al.*, 2019; Yunita *et al.*, 2011). But in reality, the problem of non-uniform induction persists. Propagation of oil palm clones through the somatic embryogenesis method indirectly begins with the callus induction stage. Callus formation is generally assisted by the addition of auxin group hormones. Auxin hormone which is often used for callus induction is 2,4-D due to its easy absorption by plants, being hardly degradable, morphogenetic activity enhancement and facilitative role in somatic cell embryogenesis (Asra *et al.*, 2020). The hormones' ability to induce callus formation is influenced by the concentration, types of explants and plants used in the culture.

The highest percentage of callus induction was produced by clone-3 (14.89%), then clone-4 (5.65%), whereas clone-1 and clone-2 produced a lower percentage of callus (around 0.45%-0.98%) (*Figure 5*). The callus induction ability of all the clones was not the same, presumably due to the genotypic factor.

Every plant has endogenous hormones present throughout its organs, of which shoots and roots generally contain the highest hormone content. Therefore, the selection of explant sources in tissue culture is very crucial. Another factor that facilitates callus induction is the synthetic hormones added to maximise callus formation since both

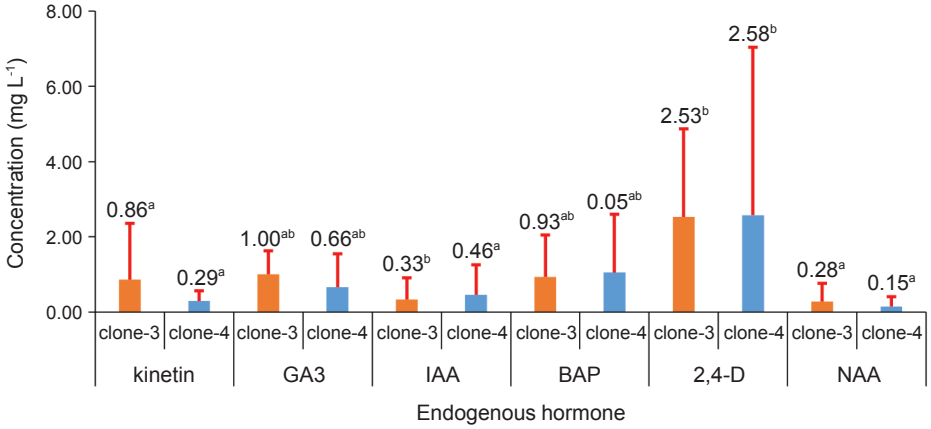

Figure 4. Endogenous hormone content of kinetin, GA_3, IAA, BAP, 2,4-D, and NAA in the oil palm young leaves of clone-1, clone-2, clone-3, and clone-4. The graph shows the mean values (±sd) of three replicates, followed by the Duncan test with a 0.05 confidence level. Numerical values with different letters indicate significant differences. (kinetin: 6-furfuryl amino purine, GA_3: gibberellic acid, IAA: indole-3-acetic acid, BAP: benzyl amino purine, 2,4-D: 2,4-dichlorophenoxyacetic acid, and NAA: naphthalene acetic acid).

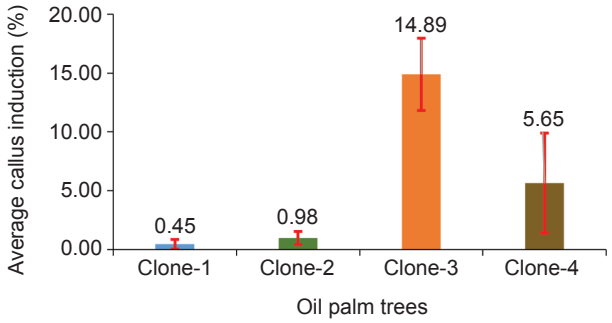

Figure 5. Percentage of callus induction of four oil palm clones.

endogenous and synthetic hormones are thought to be complementary to each other in inducing callus formation (Ayil-Gutierrez, 2013). Callus induction is generally facilitated by the hormone auxin, and 2,4-D, picloram, as well as NAA, which are the synthetic auxins commonly used in oil palm tissue culture (Reflini, 2017). Endogenous hormones zeatin and 2ip affect the formation and proliferation of embryogenic callus (Vedenicheva *et al.*, 2018). Hazelnut embryogenic callus was formed due to the balanced presence of endogenous hormones IAA, ABA, 2ip, and zeatin (Centeno *et al.*, 1997). Endogenous hormone balance between auxin and cytokinin helped the formation of *Coffea canephara* embryogenic callus (Avilez-Montalvo *et al.*, 2022). In addition to hormonal influence, callus is also formed due to a wound on the explant surface (Chen *et al.*, 2016).

The results of observations on oil palm tissue culture found that callus induction ability did not correlate with embryoid induction ability. This was presumably due to differences in callus quality produced in each clone. A quality callus is an embryogenic callus that has the potential to produce embryoids. The callus produced by clone-3 and clone-4 was more embryogenic (*Figure 6*). The embryogenic callus formed was thought to be related to the endogenous hormone content of the initial explant. Embryogenic callus had characteristics such as yellowish white colour, being solid and crumbling (*Figure 6*). Embryogenic callus colour is influenced by endogenous hormones, where low ABA concentrations affect callus colour and formation and embryoid germination (Jimenez and Bangerth, 2000; Vedenicheva *et al.*, 2018). The high endogenous hormone content of ABA in embryogenic calluses has the potential to produce embryoids and generate new plants in greater numbers. Embryogenic callus has the potential to support the mass production of oil palm ramets (Reflini, 2017).

Non-embryogenic callus was found in the induced leaves of clone-1 and clone-2, with the characteristics of being white, soft and slightly watery *(Figure 7*). Non-embryogenic callus has less potential to produce embryoids. In a previous report, the high content of endogenous hormones GA_3 and ABA coupled with low IAA had the potential to generate a non-embryogenic callus of *Ormosia henryi* Prain. culture (Wu *et al.*, 2020). High ABA content could trigger embryogenic callus formation, and low ABA concentrations increased non-embryogenic callus (Vedenicheva *et al.*, 2018). In addition to its role in callus induction and embryoid formation, the endogenous hormone ABA was also demonstrated to prevent viral attacks (Grzyb *et al.*, 2017).

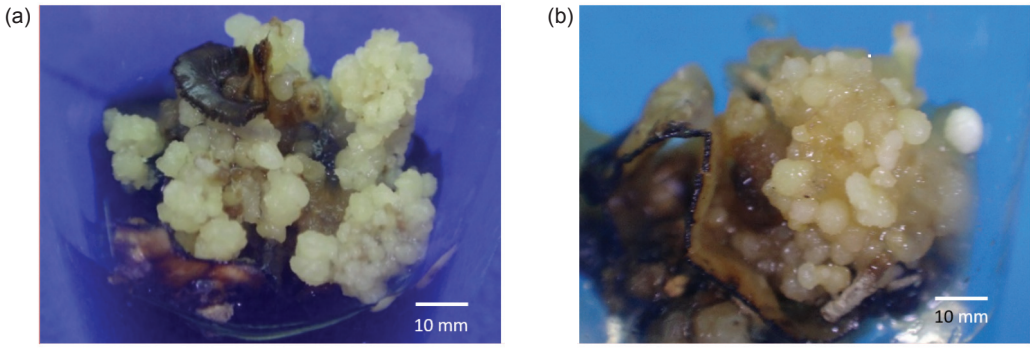

Figure 6. Leaves with embryogenic callus of (a) clone-3, and (b) clone-4.

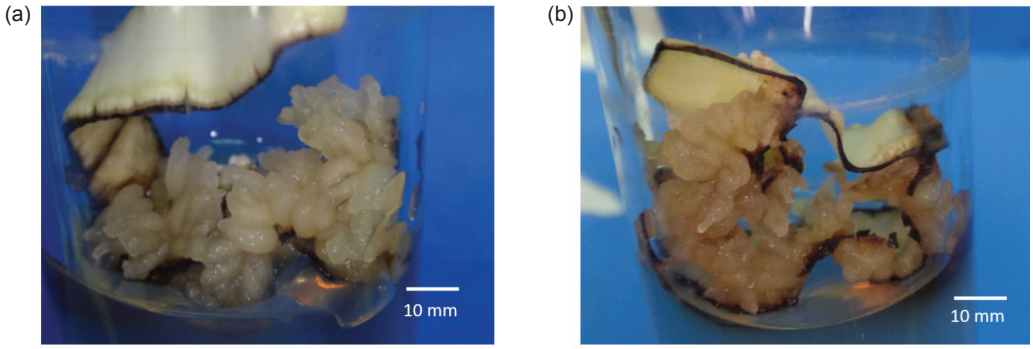

Figure 7. Leaves with non-embryogenic callus of (a) clone-1, and (b) clone-2.

TABLE 1. RESULT OF ENDOGENOUS HORMONE CORRELATION ANALYSIS ON CALLUS INDUCTION (R^2)

Endogenous hormone	Correlation value (R^2)			
	Clone-1	Clone-2	Clone-3	Clone-4
Zeatin	0.3082	0.4659	0.6218	0.3140
Kinetin			0.7500	0.7595
GA			0.2286	0.4709
IAA			0.7500	0.4395
BAP			0.6808	0.4503
ABA	0.8232	0.9371	0.0933	0.9983
IBA	0.0568	0.1702	0.0250	0.8996
2,4-D			0.3410	0.4395
NAA			0.7500	0.4395

The selection of the initial explant sample is very important because it will affect the callus induction ability and embryoid formation. Oil palm tissue culture uses samples of young leaves, which are not only merismatic but also contain endogenous hormones. In this study, the types of endogenous hormones found in clone-3 and clone-4 were more than those in clone-1 and clone-2.

The correlation of the effect of endogenous hormones on callus formation was analysed using a scatter plot in Excel, and R^2 data were obtained (*Table 1*). The results of the analysis were based on R^2 data; endogenous hormone ABA was correlated with callus formation in clone-1 (R^2; 83.32%), clone-2 (R^2; 93.71%) and clone-4 (R^2; 99.83%). Likewise, for the hormone kinetin, there was a correlation between clone-3 (R^2; 75.00%) and clone-4 (R^2; 75.95%). The correlation analysis data does not ensure that only a few endogenous hormones affect callus formation, but a combination of several other endogenous hormones had affected the formation and quality of the resulting callus.

Embryogenic calluses can be formed due to a combination of endogenous hormones and synthetic hormones added to the culture medium. The addition of synthetic hormones in culture media must be adjusted and regulated to avoid somaclonal variations. The use of synthetic hormones such as 2,4-D in high doses can cause mutations and fruit abnormalities; thus, their use must be measured in low doses (Saptari and Sumaryono, 2018). The information about the presence of endogenous 2,4-D should prevent the addition of synthetic 2,4-D at high concentrations, which can cause variations or mantled fruit formation.

Effect of Endogenous Hormones on Embryoid Induction

The resulting callus was then transferred to the embryo induction medium. In this medium, not all subcultured calluses could produce embryoids. The formation of embryoid depends on the callus cells that had formed previously. If the callus cells formed are embryogenic, the potential for embryoid formation is very high. In this study, the callus formed had the potential to produce a low number of embryoids. The percentage of induced embryoids for each clone was not the same (*Figure 8*). The callus from clone-3 and clone-4 produced embryoids, whereas the callus from clone-1 and clone-2 did not produce any embryoids. The induction ability of embryoids produced by clone-4 reached 44.45% (45 tubes) and clone-3 was about 9.49% (18 tubes) (*Figure 8*).

Oil palm somatic embryos that have the potential to differentiate into plantlets had the following characteristics: Yellowish-white, dense embryos, loose embryos (not sticking together) and regular shaped (*Figure 9*).

Endogenous hormones are believed to play a role in the formation of the embryo. The results of the analysis of young oil palm leaves of clone-3 and clone-4 revealed nine types of endogenous hormones in various concentrations. Several endogenous hormones were found to correlate with embryoid formation. Endogenous hormones zeatin (R^2; 98.93%) and ABA (R^2; 77.50%) correlated in helping the formation of embryoids in clone-3 (*Figure 10*), whereas the hormones IBA (R^2; 85.18%) and ABA (R^2; 98.70%) correlated in the formation of embryoid in clone-4 (*Figure 10*). The combination of various types of endogenous hormones collaborated in the formation of potential embryoids. The high content of ABA helped in embryo differentiation, while the addition of low IAA and zeatin maintained the embryogenic potential in Korean pine callus (Liang *et al.*, 2022). The content of endogenous hormones ABA, IAA, and GA_3 in *Ormosia henryi* Prain. decrease with the process of embryogenesis (Wu *et al.*, 2020).

The auxin and cytokinin found in clone-3 and clone-4 in combination with synthetic hormones in culture media were able to support the formation of embryogenic and embryoid calluses. The addition of synthetic hormones into the media increases the concentration of endogenous hormones in cells,

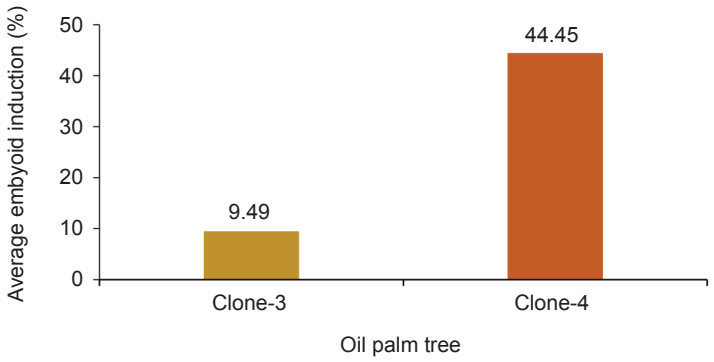

Figure 8. Percentage of somatic embryo formation from (a) clone-3, and (b) clone-4.

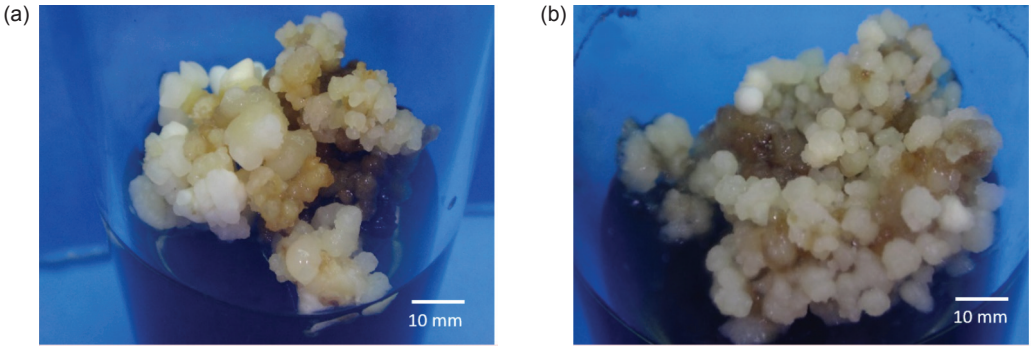

Figure 9. Embryogenic callus of prospective embryoids of (a) clone-3, and (b) clone-4.

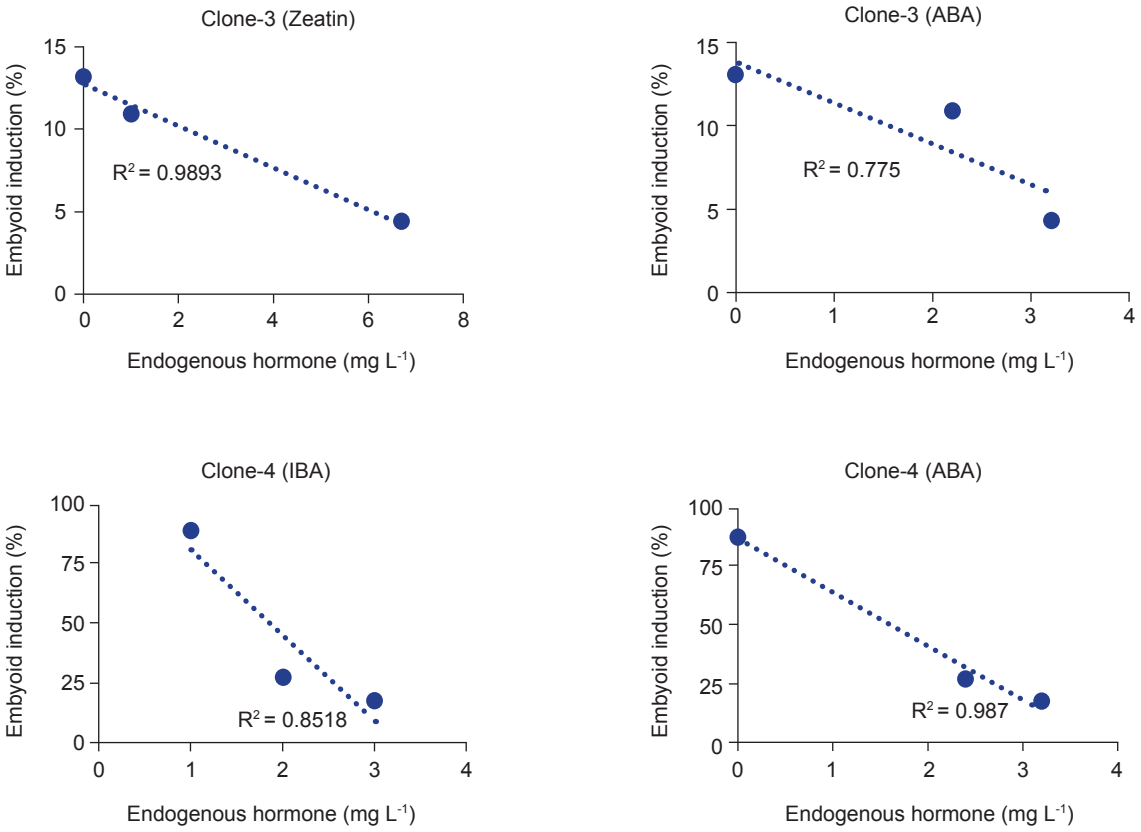

Figure 10. Correlation of endogenous hormones with the percentage of embryoid formation in clone-3, and clone-4.

hence triggering tissue growth and development (Gaba, 2005). Cytokinin hormone is needed in the formation of embryoids. The types of cytokinin added to oil palm tissue culture media were BAP, 2iP and kinetin (Weckx *et al*., 2019). Cytokinin hormones also promote the regeneration of embryos into shoots and plantlets (Aslam *et al*., 2011). The addition of BAP-type cytokinins at a concentration of 1 M was able to increase the germination and development of somatic embryos (de Olivera *et al*., 2016).

The addition of synthetic hormones into the culture medium must consider the presence of endogenous hormones in the selected explant source. Excessive accumulation of hormones in plant tissues and culture media can cause abnormalities problems (Weckx *et al*., 2019). Growth regulators or hormones do not have a direct effect as mutagens; however, if added excessively, they can stimulate fast irregular growth, causing abnormalities (Bairu *et al*., 2011). Other factors that can trigger abnormalities are too high concentrations of 2,4-D and repeated subculture processes (Ernayunita *et al*., 2017). In addition to functioning as a growth regulator, 2,4-D turned out to be toxic to plants when used in high concentrations (Vasil and Vasil, 1972). The use of high 2,4-D can cause physiological and biochemical disturbances in plants resulting in abnormalities. Thus, the supplementation of synthetic hormones must be carefully measured because it can trigger abnormalities when combined with high content of endogenous hormones. Initial information about the content of endogenous hormones is important; this is because the process of *in vitro* propagation of oil palm plants takes a long time. The sampling of young leaf explants to produce plantlets takes about 3-4 years. This research is expected to provide information in determining the type of hormone and the concentration of synthetic hormone needed, especially in the oil palm clonal propagation activities.

CONCLUSION

The type and content of endogenous hormones in the young leaves of each oil palm clone were found to be different. Endogenous hormones in young oil palm leaves affected the callus and embryoid induction ability. The analysis of young leaves from clone-1 and clone-2 showed three types of endogenous hormones (zeatin, ABA and IBA), whereas clone-3 and clone-4 contained nine types of endogenous hormones (zeatin, kinetin, GA_3, IAA, BAP, ABA, IBA, 2,4-D and NAA). Young explants of oil palm leaves containing high zeatin and ABA triggered the formation of non-embryogenic callus, while the young leaves containing 2,4-D and BAP demonstrated the potential to produce embryogenic callus. The presence of endogenous hormone 2,4-D in leaf explants and the addition of synthetic hormone 2,4-D around 0.002-11.050 mg L^{-1} in culture media induced the formation of embryogenic callus and had the potential to form embryoids and produce plantlets. The right and measurable combination of endogenous and synthetic hormones can support the clonal production of oil palm.

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