IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. IV (Sep. – Oct. 2017), PP 43-50
www.iosrjournals.org

The Effect of Adding 2, 4 – *Diclorophenoxyacetic Acid* (2,4-D) on The Genetic Changes Organogenesis of Oil Palm based on *Simple Sequence Repeats* (SSR) Markers

*Lidya Wardani
(Post Graduate Univeristy of Nort Sumatra)
Corresponding Author: Lidya Wardani

Abstract: *The effect of adding 2,4 – Diclorophenoxyacetic Acid (2,4-D) On The genetic changes organogenesis of oil palm based on Simple Sequence Repeats (SSR) markers has been done at Genetic Laboratory and Plant Tissue Culture of Faculty Mathematics and Science, University of Sumatera Utara. The experimental design was completely randomized with four levels of 2,4-D concentrations; 0 mg/L, 100 mg/L, 115 mg/L, 120 mg/L, 135 mg/L, and 140 mg/L. The statistical analysis showed that 100 mg/L of 2,4-D significantly affected the initiation organ. The SSR markers with 8 primer showed that initiation 2,4-D caused genetic changes organogenesis of oil palm.*

Keywords - *2, 4-D, organogenesis, genetic changes, oil palm, SSR*

Date of Submission: 16-09-2017 Date of acceptance: 28-09-2017

I. Introduction

Palm oil (Elaeis guineensis Jacq.) is one of the oldest crops grown as a plantation crop originating in Africa and America. Indonesia is one of the Southeast Asian countries that makes palm oil plant as a commercial plant. Oil palm plays an important role in contributing significantly to increase state revenues and drive development (BAPENAS, 2010). In 2010, Indonesia occupied the first position in palm oil production with increasing plantation area of up to 7.8 million hectares.

Propagation of palm oil through tissue culture is one of the most potential approaches to meeting the demand for superior seeds. Oil palm tissue culture can be done through organogenesis and embryogenesis. Organogenesis is the process of development of shoots or root adventive from within the callus cells. Organogenesis process can occur directly by explant initiation and indirectly through initiation of callus that formed during embryogenesis. Production of embryogenesis that forms globular cells will develop into primordium shoots or roots and will form the plantlet [1]. According to author [2], suspension culture is a cell aggregate in aqueous medium by aeration which is initiated from a crumbling callus. Growth in cell suspension cultures in liquid media grows faster and is easier to control with substitution or addition of media than callus cultures grown in solid media [3].

Oil palm clones produced from tissue culture techniques generally occur 10-40% change in the direction of abnormalities in reproductive organs i.e. flowers and fruits that can reduce the production of palm oil. This process of abnormality occurs in the conversion of one or more primordial anther into a soft addition carpel and develops into a mantle [4]. Cell cultures can induce genetic or epigenetic variations resulting in soma clonal variation. Genetic diversity in explants is caused by mutated cells. The use of 2,4-dichlorophenoxyacetic acid (2,4-D) and the selection of plant regulators (ZPTs) used in high concentrations increased the frequency of abnormally grown regenerates crops, callus calling phases and excessive subculture subcultures also contributed to the formation of soma clonal variation [5]. While Bayzura reported that the use of 2,4-D at certain concentrations of palm oil callus cultures can cause genetic changes of calluses [6]

Genetic variations can be detected with genetic markers such as Simple Sequence Repeaters (SSR). SSR is a common molecular marker used for genetic markers because it is reproducible, colonized and can detect high allele variations [7]. SSR markers for oil palm are the most prospective markers for analyzing the population and knowing the genetic structure of oil palm [8], while Zulhermana said that based on this, it is necessary to conduct research to find out the genetic variation of organogenesis from culture of oil palm suspension with marker of Simple Sequence Repeaters (SSR) [9]. Therefore it is necessary to know the effect of adding 2,4-D which influence to genetic change in oil palm culture. In this research, palm culturing is done at different concentration levels of ZPT (2,4-D).

II. Method

The research method used is experiment research method with qualitative description approach. This test is performed to determine the quality of DNA obtained so that it can be used for PCR process. The DNA quality test was determined using electrophoresis (C. Scientific EPS-300X). DNA isolation in this study was based on the modified CTAB [10] method. 0.4 g explant crushed in porcelain with the help of liquid nitrogen. DNA quantity test was performed by measuring the concentration and purity of DNA using Nano photometer (IMPLEN P-360 Nano Photometer P-Class). While for binary data DNA tape is processed with the help of program Numerical Taxonomy and Multivariate Analysis System (NTSYS) ver. 2.11a. The results of this analysis are presented in the form of genogram trees. And to determine the level of primary information, a calculation of Polymorphic Information Content (PIC) is performed. The PIC is calculated for each SSR marker [11].

III. Discussion And Results

3.1 Discussion

3.1.1 Procedure

Equipment used is water bath, autoclave, laminar air flow, hot plate, glassware, freezer, PCR, electrophoresis, Eppendorf, Nano photometer, centrifugation, micro pipette, vortex, 2 ml micro tube, pestle and mortar, water bath. Explants used in this study were obtained from shoot buds (apical bud) of palm oil, varieties of tenera 2.5 years old. The chemicals used are the components of the Y3 (Eeuwens), growth regulator 2,4 - dichlorophenoxyacetic acid (2,4 - D), CTAB extraction buffer (100 mM Tris HCL, 4M Nacl, 20 mM EDTA, 3% CTAB, 6% PVP and 0.2% β-mercaptoetanol), Chloroform, Isoamilalkohol, Isopropanol, Alcohol 70%, 5 M NaCl, Sodium acetate, Phenol, TE Buffer, TAE 1X, Agarose, Promega GoTaq® Green Master Mix, sterile ddH20, primary SSR, 1% Ethium bromide, Loading dye 6X, RNA-se. Sterilization using ascorbic acid, sodium hypochlorite, tween® 20 and detergent.

Table 1. shows treatment of Y3 medium with different concentration of growth regulator 2,4 - dichlorophenoxyacetic acid (2,4-D).The tools used in this study were washed with detergent and rinsed with running water, then the apparatus was dried and sterilized in an autoclave at a temperature of 1210C with a pressure of 17.5 psi for 30 minutes. Along with the tools, also included bottles containing distilled water and media for sterilization. The medium used is the Y3 medium (Eeuwens, 1976) with the addition of ZPT 2,4 - D hormone with different concentrations. Each macro, micro, and vitamin nutrient elements were dissolved in accordance with the order of medium Y3 (composition of medium Y3 in appendix 1), then adding sugar and Fe EDTA as needed. The media were then given a growth regulator substance of 2,4-D according to the treatment and the acidity of the media was measured using a pH meter of about 5.8. Furthermore the medium may be stored in the culture room at 25 ° C for 1 week before use

3.1.2 Isolation of DNA

DNA isolation in this study was based on the modified CTAB (Doyle & Doyle, 1990) method. 0.4 g explant crushed in porcelain with the help of liquid nitrogen. Entered smooth explant like white flour into 2 ml Eppendorf containing 1 ml warm extraction buffer (60oC) containing 1.5% CTAB (w / v) (75 mM Tris-HCl pH 8, 1.05 M NaCl , 15 mM Na2EDTA) and added 2% β-mercaptoethanol, 1% PVP. The suspension was incubated at 60 ° C for 30 minutes with a note every 10 minutes the sample was removed, homogenized by inverted, after incubation, the explant was allowed to cool at room temperature and continued by purification process.

The purification process begins by adding chloroform: isoamilalcohol (24: 1) in the same volume and divortext to homogeneous, then centrifuged (Eppendorf centrifuge 5430R) at 10,000 rpm at 4 ° C for 5 minutes. The supernatant was transferred to a new 1.5 ml micro tube and added 1 ml of cold isopropanol at -20 ° C. Then homogenized and centrifuged at the same rate of 10,000 rpm at 4 ° C for 5 minutes. The supernatant was taken with a pipette and transferred to a new sterile 1.5 ml micro tube. DNA was precipitated by addition of 1/10 times the volume of sodium acetate (pH 5.2) and 3 times the absolute cold ethanol volume, then homogenized. DNA was incubated at -20 ° C for 1 night and then centrifuged at 10,000 rpm at 4 ° C for 15 minutes. The supernatant is removed while the pellets are removed and cleaned with 70% alcohol twice. The next stage of DNA is dried at room temperature with an upside-down tube position. Dried DNA was dissolved with sterile aqua bides (ddH2O) of 100 µL. The RNA contaminant in DNA was removed by adding 5 µL RNase (20 mg / ml) and incubated at 37 ° C for 90 min. The RNase is disabled by incubation at 70 ° C for 3 minutes and stored at -20 ° C for subsequent use.

DNA Quality and Quantity Test This test is performed to determine the quality of DNA obtained so that it can be used for PCR process. The DNA quality test was determined using electrophoresis (C. Scientific EPS-300X) using 0.8% agarose gel. (0.8 grams of agarose in 100 ml of TAE 1X buffer) for 30 min at 70 V and 100 mA. The electrophoresis results were stained by immersion in an ethidium bromide solution (EtBR 1%) for 10 minutes then immersed in aquades for 5 minutes. The quality of electrophoretic DNA was observed under

the UV transluminator (G.BOX SYNGENE) and documented by Kodak gel logic with the software. DNA quantity test was performed by measuring the concentration and purity of DNA using Nano photometer (IMPLEN P-360 Nano Photometer P-Class). DNA concentration was calculated by the formula A260 x 50 x dilution factor. As for the measurement of DNA purity is done by comparing absorbance 260/280 (A260 / 280). The pure DNA has A260 / 280 = 1.8 to 2.0.

3.1.3 DNA amplification with SSR markers

The PCR reaction is performed in a PCR (Polymerase Chain Reaction) machine using the Go Taq® Green Master Mix Promega kit which consern with Table 2. The eight primary SSRs used refer to the research that has been done by Billote et al. (2001) in detecting genetic diversity of oil palm clones.

Based on the table that the amplification reaction is further carried out by inserting the tube containing the material for PCR reaction into the PCR (Eppendorf vapo protect) machine block with the time used is: denaturation of 95° C for 30 seconds, then denaturation 95° C for 1 minute, annealing (Primary optimum temperature) 55^{o} C and 58° C for 30 seconds, and extension at 72° C for 1 minute, followed by the final extension at 72° C for 5 minutes, cooling after the cycle is completed at 4° C. The PCR reaction was performed for 35 cycles. The result of PCR reaction was electrophoresed on 2% agarose gel. Electrophoresis was carried out for 90 min at a voltage of 70 V, 100 mA at room temperature. The electrophoresis staining was done by soaking agarose in ethidium bromide solution (EtBR 1%) for 10 minutes. The dyed gel was soaked in sterile aquades for 3 minutes and continued observation of the amplified band using the UV transiluminator (G.BOX SYNGENE).

3.1.4 Polymorphic Band of DNA

PCR DNA is translated into binary data based on the presence or absence of a tape, provided that the value 0 (zero) for no band, and the value of 1 (one) for the existence of the band in a similar position of each individual being compared. The way of scoring can be seen in Figure 1. From the table can be seen the development of organ formation through embryogenic callus initiation given ZPT 2,4-D with different concentration showed the development of uneven organ formation. The organs begin to form on the 105th day after planting, marked with a white root shoot (Appendix 3). Balzon et al., (2013) states that the division of embryonic callus cells can affect the formation of organs by forming somatic embryo cells that can differentiate into organs. The control treatment without ZPT (0 mg/L) does not initiate the presence of only swelling explant. It is suspected that the lack of endogenous hormone supply present in explants and the components contained in the media have not been able to induce the formation of organs. The association of the time of the formation of organs with the 2.4-D growth regulator treatment can be seen in Figure 2.

From the Figure 2 shows that explant given the concentration of 2,4-D 100 mg / L (P1) is the most rapid treatment induce organ growth that is 105 days after planting. Treatment (P2) with a concentration of 2,4-D showed organ growth at 153 days after planting. Treatment (P3) with a concentration of 2,4-D showed organ growth at 120 days after planting. Treatment (P4) with concentration 2,4-D showed organ growth at 134 days after planting Treatment (P5) with concentration 2,4-D 140 mg / L showed organ growth that is 114 days after planting. This suggests that the sugar component as well as the 2.4-D concentration contained in the medium can affect the growth and development of somatic embryos to form organs in suspension cultures. Yelnitis & Komar research (2010) states that the higher concentrations of 2,4-D used faster the callus induction than the treated explants without ZPT 2,4-D showed only thickening and did not develop into callus in the long term.

3.2 Result

3.2.1 DNA Isolation Results

The qualitative test of isolation from 6 samples of palm oil DNA was performed by using electrophoresis on 0.ose of agarose gel. This test is performed to determine the quality of DNA obtained so that it can be used for PCR process. In electroforegram on Fig. 3 shows the existence of one whole band of gel wells, indicating that the band is good and can be selected for further analysis.

Whole DNA is characterized by the absence of a smear. Smears are formed by degradation of DNA into short pieces or because of contamination. In the PCR process, whole DNA will give more accurate results (Syafaruddin & Santoso, 2011). The size of the obtained DNA band is above 10000 bp. Quantitative DNA test was performed using Nano photometer (IMPLEN P-360 Nano Photometer P-Class) to obtain the value of purity and concentration (Table 4.1). The purity value of DNA obtained ranged from 1.817 to 2.615. According to Sambrook & Russell (1989), the purity limit commonly used in molecular analysis at A260 / A280 ratio is 1.8 - 2.0. A purity value greater than 2.0 indicates the presence of RNA contaminants, whereas a purity value less than 1.8 indicates the presence of a protein contaminant (Husniyati, 2012).

3.2.2 Analysis of Genetic Changes

The amplified band analysis of 6 palm oil DNA samples using 8 SSR markers shows a ribbon pattern with varying polymorphisms. The SSR markers tested showed high polymorphism with an average value of PIC (Polymorphism Information Content) 0.81. The PIC values and the number of alleles detected in this study were relatively high compared with other studies and appear on Table 3. In the study of Tasma et al. (2013) of the accession of palm oil from Cameroon by using 12 SSR markers it is known that the average PIC value is 0.53 with an average number of alleles of 3.6 per SSR mark.

In the study of Billotte et al. (2001) to palm oil using SSR marks indicates PIC values above 60% and number of alleles per locus of more than 5 alleles per SSR locus. According to Hartati et al. (2010) states that the standard microsatellite marker that can be used for genetic diversity analysis is that each locus must have more than 4 alleles with PIC values of each locus over 0.7 and allel sizes ranging from 100-300 bp. According to Tasma et al. (2013) PIC values and number of alleles per locus are highly dependent on the diversity of germplasm accessions tested and characteristics of SSR markers used.

DNA amplification with PCR

The pure DNA was amplified by SSR technique using 8 primers and produced a polymorphic band pattern. At 8 primers showed a genetic change from the parent (P0) to tissue culture results with a given concentration of 2,4-D. Genetic change is indicated by the presence or absence of DNA bands of a certain size as on Figure 4.

The result of DNA amplification by giving 2,4-D at treatment of P1 (100 mg / L) showed a genetic change from parent (P0) to result of treatment of P1 (100 mg / L) with 8 primer yield 11 bands. In the 100 bp range there is a tape in the parent plant (P0), but the reduction in the number of bands in the treatment of P1 (100 mg / L) found in the primers EgCIR008 (b), EgCIR0446 (c), EgCIR0337 (d), EgCIR0409 (f) , EgCIR0905 (g) and EgCIR0465 (h). In the 200 bp range, DNA bands appear on the parent plant (P0) but in the primary EgCIR0243 (a), EgCIR008 (b), EgCIR0337 (d), EgCIR0409 (f), and EgCIR0465 (h) absence of bands appears. There is an addition of 1 band of approximately 254 bp which appears on the parent plant (P0), but on the treatment of P1 (100 mg / L) on the primer EgCIR0243 (a), EgCIR008 (b), EgCIR0446 (c), EgCIR0337 (d) EgCIR0781 (e), EgCIR0409 (f), EgCIR0905 (g) and EgCIR0465 (h) absence of band appears. Ribbon reduction also occurs in the 300 bp range that appears in the parent treatment (P0) at 8 primers, but on treatment P1 (100 mg / L) with the primers EgCIR0446 (c) and EgCIR0781 (e) absence of band appears.

The result of DNA amplification by giving 2,4-D at P2 treatment (115 mg / L) showed the genetic change from parent (P0) to treatment P2 (115 mg / L) with 8 primer yield 14 bands. In the 100 bp range there is a tape in the parent plant (P0), but at treatment P2 (115 mg / L) in the primers EgCIR008 (b), EgCIR0781 (e), and EgCIR0905 (g) absence of band appears. Ribbon reduction also occurs in the range of 200-300 bp occurring in the parent plant (P0), but at treatment P2 (115 mg / L) in the primer EgCIR0337 (d) absence of band appears. There was addition of 1 band of about 400 bp at treatment P2 (115 mg / L) on primer EgCIR0781 (e), but on parent plant (P0) on 8 primary absence of band appears.

Differences in the number of bands produced indicate the possibility of changes in DNA sequences in the sample of oil palm culture. Generally, genetic changes in palm culture lead to DNA methylation causing morphological changes in the palm oil flower organ. The number of DNA bands detected in each primer depends on the base order of the primers and the presence or absence of variations in the particular genotype. In this study used the primary type of SSR which is a primary microsatellite is a primer designed from the area of microsatellite which is a sequence repeated. The number of bands produced by each primer depends on the distribution of homologous sites with the primary sequence in the microsatellite region.

3.2.3 Dendogram analysis

The genetic relationship of each sample of oil palm culture in this study can be determined based on the genetic similarity between individual plants by comparing the SSR bands that have been produced. The genetic changes occurring in 6 amplified palm oil cultures using 8 SSR primers can be seen in the genetic similarity dendogram on Figure

Based on Figure 5 shows the dendogram it can be seen that no treatment has a genetic similarity with the control (parent) of 100%. This indicates that there has been a genetic change from the host to the result of tissue culture. In the genetic similarity coefficient 0.59 (59%) the oil palm culture is divided into two groups. Group I was the control (P0) in which there was no addition of 2,4-D concentration whereas group II consisted of treatment (P1-P5) of addition of 2,4-D concentration. This shows that the genetic difference between control (P0) and treatment (P1-P5) is 41%. According to Karp (1995), the number of factors affecting genetic changes, namely the source of explant, the selection of growth regulatory substances used and the number of subcultures performed during the multiplication of embryo somatic cells and maintenance phase.

IV. Figures and Tables

Table 1: Treatment of Y3 medium with different concentration of growth regulator 2,4 - dichlorophenoxyacetic acid (2,4-D).

No.	Code	Plainting
1.	P0	Explant Plainted on Media Y3 + 0 mg/L (Control)
2.	P1	Explant Plainted on Media Y3 + 100 mg/L 2,4-D
3.	P2	Explant Plainted on Media Y3 + 115 mg/L 2,4-D
4.	P3	Explant Plainted on Media Y3 + 120 mg/L 2,4-D
5.	P4	Explant Plainted on Media Y3 + 135 mg/L 2,4-D
6.	P5	Explant Plainted on Media Y3 + 140 mg/L 2,4-D

Number of treatments : 6
Number of repetitions : 5
Total of treatment units : 6 x 5 = 30 units

Table 2: Material for one-off PCR reactions

No.	Component of PCR	Volume
1.	GoTaq® Green Master Mix, 2X	5 µl
2.	DNA template (<250 ng)	1 µl
3.	Primer R (10 pmol/µl)	1 µl
	Primer F (10 pmol/µl)	1 µl
4.	*Nuclease free water*	2 µl
	Final Volum	10 µl

Table 3: Some Primer for SSR Primer

No.	Nama Primer	Repeat Motif	Sequent Primer Forward (5' ---------- 3') Reverse (5' ---------- 3')
1.	EgCIR0243	(GA)17	F : TGGAACTCCTATTTTACTGA R : GCCTCGTAATCCTTGTCA
2.	EgCIR008	(GA)17	F : CGGAAAGAGGGAAGATG R : ACCTTGATGATTGATGTGA
3.	EgCIR0446	(CCG)7	F : CCCCTTCGAATCCACTAT R : CAAATCCGACAAATCAAC
4.	EgCIR0337	(GT)6 (GC)4	F : GTCTGCTAAAACATCAACTG R : GAGGAGGAGGGGAACGATAA
5.	EgCIR0781	(GA)17	F : CCCCTCCCTACCACGTTCCA R : TGTTTGCTGTTGCTCTTTGATTTC
6.	EgCIR0409	(CCG)6	F : AGGGAATTGGAAGAAAAGAAAG R : TCCTGAGCTGGGGTGGTC
7.	EgCIR0905	(GT)14ctca (GA)11	F : CACCACATGAAGCAAGCAGT R : CCTACCACAACCCCAGTCTC
8.	EgCIR0465	(CCG)6	F : TCCCCCACGACCCATTC R : GGCAGGAGAGGCAGCATTC

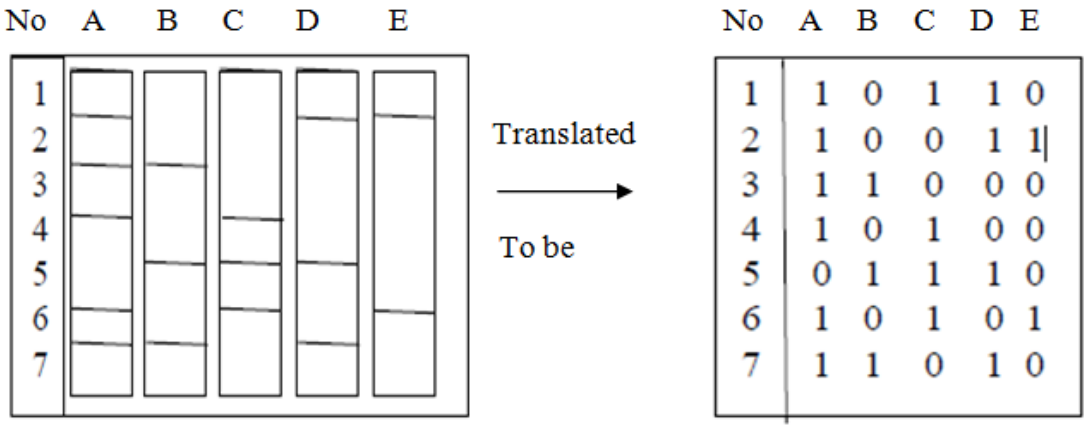

Fig 1: DNA Ribbon Translation Patterns

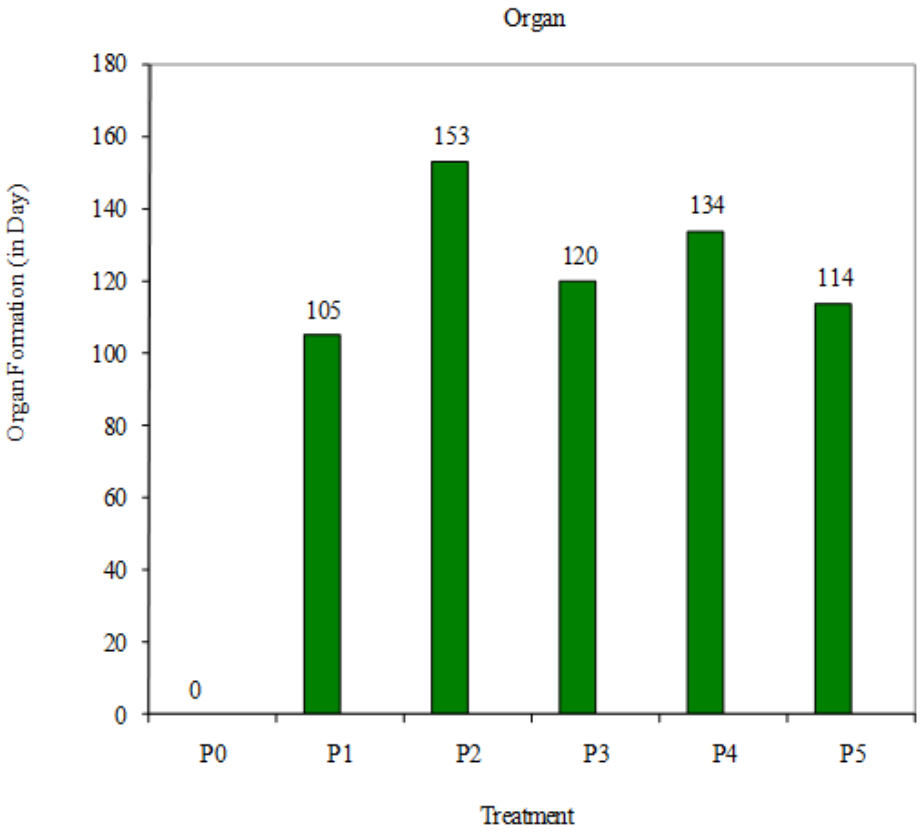

Fig. 2: Time of Establishment of Organ with Treatment of Growing Substance (2.4-D).

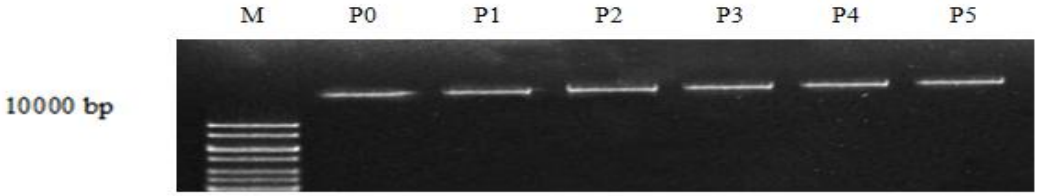

Figure 3: Genomic DNA electrophoresis of 6 Palm Oil Samples in Gel Agarose 0.8%. M = Marker 1 Kb, P0 = Y3 + 0 mg / L (control), P1 = Y3 + 100 mg / L 2,4-D, P2 = Y3 + 115 mg / L 2,4-D, P3 = Y3 + 120 mg / L 2,4-D, P4 = Y3 + 135 mg / L 2,4-D, P5 = Y3 + 140 mg / L 2,4-D.

Table 4: Purity and DNA Concentration

Example Code	Puirty (A260/280)	Consentration (ng/µl)
P0	2,134	71,5
P1	2,615	117,0
P2	2,055	75,0
P3	2,107	118,0
P4	2,219	178,0
P5	1,871	74,5

Table 5: Number of Bands of DNA Amplification Results with 8 Primer SSR

No.	Primer Name	Pita Size (bp)	Pita-Pita		Value PIC
			Pattern Pita	Polimorfisme	
1.	EgCIR0243	18-300	4	3	0,67
2.	EgCIR008	91-215	3	2	0,70
3.	EgCIR0446	141-322	5	5	0,90
4.	EgCIR0337	146-331	4	4	0,92
5.	EgCIR0781	100-886	6	5	0,86
6.	EgCIR0409	103-285	5	5	0,88
7.	EgCIR0905	147-295	4	4	0,80
8.	EgCIR0465	119-281	3	3	0,81
	Total		34	31	6,55
	Average	18-886	4,25	0,91	0,81

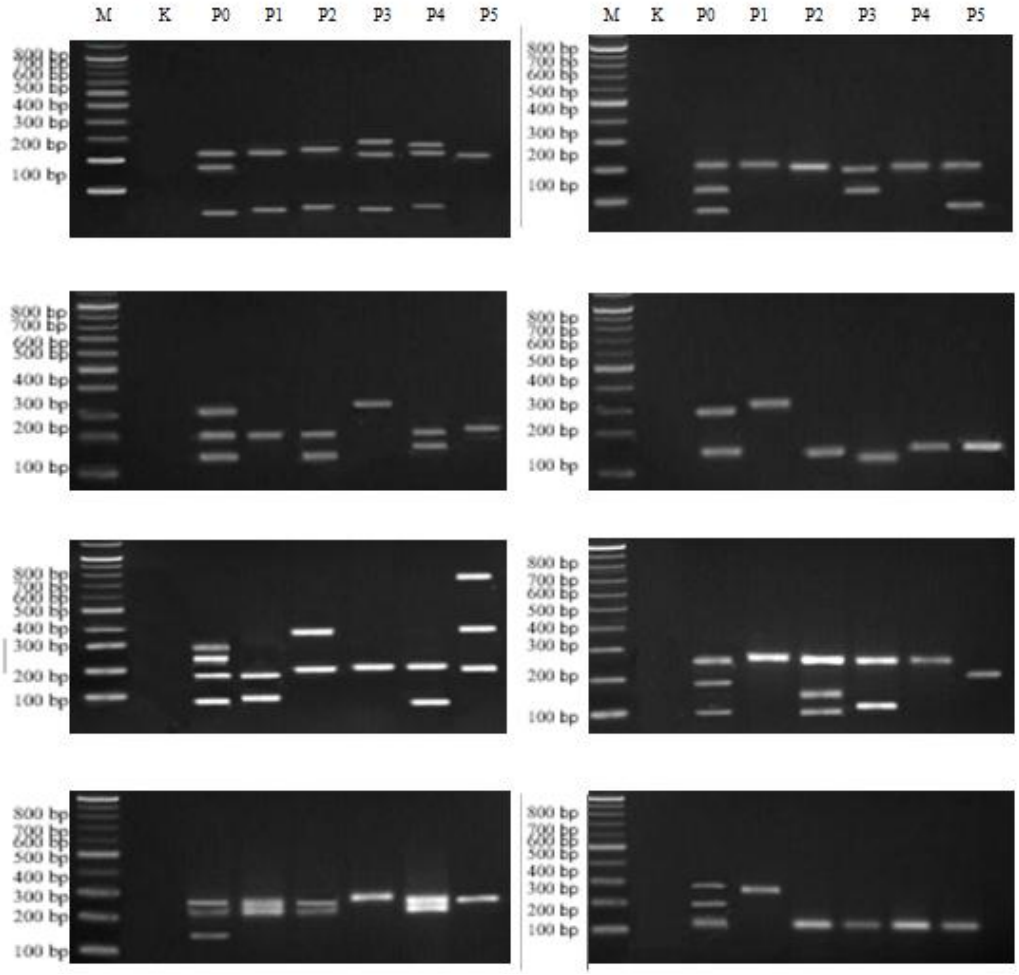

Figure 4: The SSR tape profile using the primary EgCIR0243 (a), EgCIR008 (b), EgCIR0446 (c), EgCIR0337 (d), EgCIR0781 (e), EgCIR0409 (f), EgCIR0905 (g), EgCIR0465 (h). M = Marker, P0 = Y3 + 0 mg / L (control), P1 = Y3 + 100 mg / L 2,4-D, P2 = Y3 + 115 mg / L 2,4-D, P3 = Y3 + 120 mg / L 2,4-D, P4 = Y3 + 135 mg / L 2,4-D, P5 = Y3 + 140 mg / L 2,4-D.

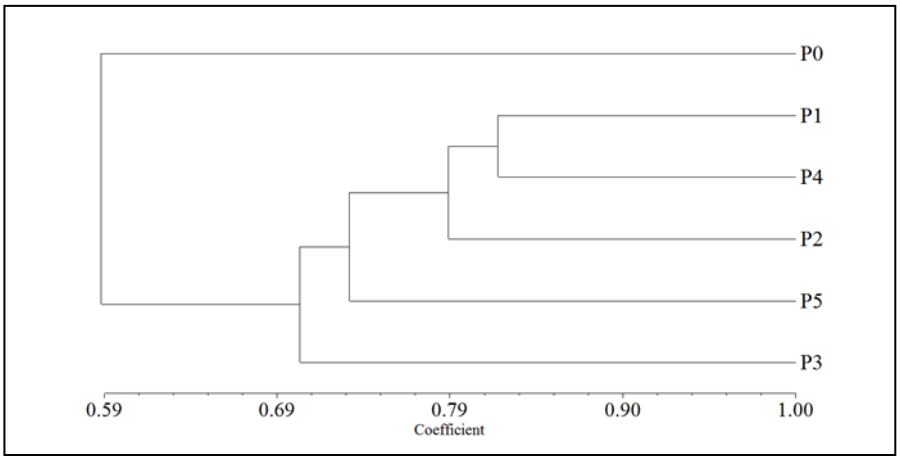

Figure 5: Dendogram genetic similarity of oil palm culture based on 8 SSR primers

V. Conclusion

From the results of this study can be concluded that:

a. The fastest initiation time of palm organ at treatment of P1 (100 mg / L 2,4-D) is for 105 days.

b. Differences in the pattern of DNA bands amplified by 8 primers (EgCIR0243, EgCIR008, EgCIR0446, EgCIR0337, EgCIR0781, EgCIR0409, EgCIR0905, and EgCIR0465) show genetic changes in palm organogenesis caused by 2.4-D concentration treatment.

Further research is needed to see the genetic diversity of cultures by using different concentrations and different primers or by using different marker techniques.

References

[1]. Purnamaningsih, R. 2002. Regenerasi Tanaman Melalui Embryogenesis Somatik Dan Beberapa Gen Yang Mengendalikannya. Buletin Agrobio, 5 (2) : 51 – 58.
[2]. Zulkarnain. 2009. Kultur Jaringan Tanaman (Solusi Perbanyakan Tanaman Budidaya). Jakarta: Penerbit Bumi Aksara.
[3]. Hutami. 2009. Tinjauan Penggunaan Suspensi Sel dalam Kultur In Vitro. Jurnal AgroBiogen 5(2): 84-92.
[4]. Corley, R. H. V., Lee, C. H., Law, L. H., and Wong, C. Y. 1986. Abnormal Fower Development in Oil Palm Clones. Planter. 62: 233 – 240.
[5]. Karp, A. 1995. Somaclonal variation in crop improvement. Euphytica, 185:295-302.
[6]. Bayzura, Elva Nuriza. 2014. Pengaruh 2,4-D dan Frekuensi Subkultur Terhadap Perubahan Genetik Kultur Apical Bud Kelapa Sawit. [Skripsi]. Medan: Universitas Sumatera Utara.
[7]. Utami, E.S.W., Issirep, S., Taryono, Endang, S. 2007. Pengaruh α-Napthaleneacetic Acid (NAA) Terhadap Embriogenesis Somatik Anggrek Bulan Phalaenopsis amabilis (L.) BI. Biodiversitas 8(4): 295 – 299.
[8]. Singh, R., M.Z. Noorhariza, N.C. Ting, R. Rozana, S.G. Tan, L.E.T. Low, M. Ithnin, and S.C. Cheah. 2008. Exploiting an oil palm EST the development of gene-derived and their exploitation for assessment of genetic diversity. Biologia. 63: 1–9.
[9]. Zulhermana. 2009. Keragaman Genetik Intra dan Interpopulasi Kelapa Sawit (Elaeis guineensis Jacq.) Pisifera Asal Nigeria Berdasarkan Analisis Marka Simple Sequence Repeat (SSR). [Tesis]. Bogor: Institut Pertanian Bogor.
[10]. Doyle, J.J. and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.
[11]. Smith dl, krikorian AD. 2000. pH control of carrot somatic embryogenesis. N: Nijkamp HJJ, van der Plas LHW, Van Aartrijk J (eds). Progress in Plant Cellular and Moleculer Biology (pp. 449-453). Kluwer Academic Publisher, Dordrecht.

Lidya Wardani. "The Effect of Adding 2, 4 – Diclorophenoxyacetic Acid (2,4-D) on The Genetic Changes Organogenesis of Oil Palm based on Simple Sequence Repeats (SSR) Markers." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 5, 2017, pp. 43–50.