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PROLIFERATION OF OIL PALM (*Elaeis guineensis* Jacq.) EMBRYOGENIC CALLUS WITH REPEATED SUBCULTURES IN LIQUID MEDIUM

Perbanyakan Kalus Embriogenik Kelapa Sawit (*Elaeis guineensis* Jacq.) dengan Subkultur Berulang dalam Media Cair

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ABSTRACT
The availability of high-quality seeds is now a necessity. This is due to a government program to replace oil palm trees in smallholder plantations with high quality seeds. An efficient protocol to produce a large number of embryos is needed. To increase the number of embryogenic callus production, the callus proliferation experiment was carried out through suspension culture. This study aimed to examine the proliferation ability of embryogenic callus from three different oil palm clones, in several repeated subcultures. Liquid MS media added with 1 ppm 2.4-D and 0.1 ppm NAA were used. Embryogenic callus was weighed by 0.1–0.2 g, transferred into the liquid media, shaking at 60–80 rpm and 27 ºC for 8 weeks without light. Continues subcultures were repeated up to 7 times. The results showed that the growth rate of embryogenic callus increased in the third and fourth subcultures and then decreased in subsequent subcultures. It also revealed that the entire embryogenic callus from the first subculture up to seventh subculture still has the ability to regenerate into new plants. These results indicate that oil palm embryogenic callus can be proliferated by suspension culture with a limit up to the fourth subculture.

Keywords: *embryogenesis, oil palm clone, proliferation, somatic, suspension culture*

ABSTRAK
Ketersediaan benih kelapa sawit berkualitas saat ini merupakan kebutuhan karena adanya program pemerintah untuk menggantikan tanaman sawit di kebun-kebun petani. Salah satu cara vegetatif yang dapat dilakukan adalah meningkatkan jumlah kalus embriogenik yang dihasilkan melalui pengembangan kultur suspensi. Penelitian ini bertujuan mengkaji kemampuan proliferasi kalus embriogenik dari tiga klon kelapa sawit, pada beberapa kali subkultur yang berulang. Media cair MS dengan penambahan 1 ppm 2,4-D dan 0,1 ppm NAA digunakan untuk memperbanyak 0,1–0,2 g kalus embriogenik, dikocok pada 60–80 rpm dan suhu 27 ºC tanpa cahaya selama 8 minggu. Subkultur berulang dilakukan hingga 7 kali. Hasil percobaan menunjukkan bahwa kemampuan proliferasi kalus dipengaruhi oleh genotip tanaman induk. Rata-rata kalus embriogenik dapat meningkat pada subkultur ke-3 dan ke-4 dan semakin menurun pada subkultur selanjutnya. Kalus embriogenik hasil proliferasi subkultur pertama hingga ke-7 dapat tumbuh menjadi calon tanaman baru. Hasil ini menunjukkan bahwa kalus embriogenik kelapa sawit dapat diperbanyak dengan kultur suspensi pada batas sampai subkultur ke-4.

Kata Kunci: embriogenesis, somatik, klon, kultur suspensi, proliferasi

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is one of the agriculture commodities that has an important role in the Indonesian economy. In 2018, Indonesia's trade balance recorded a surplus of 16.5 billion USD for CPO (crude palm oil) and its derivatives, and 1.8 billion USD for PKO (palm kernel oil) and its derivatives. This makes palm oil one of the highest income contributors in Indonesia (Directorate General of Estate Crops 2019). Oil palm fruit can produce vegetable oil (CPO) either from mesocarp or kernel. Both of them are widely used as food, medicine, cosmetics and bioenergy. Data collection on oil palm plantation area actually recorded around 16,381,959 hectares (Ministry of Agriculture RI 2019). In fact, the area of oil palm plantations continues to increase every year, and to hold back the expansion of plantations, restructuring has begun to be carried out by limiting the increase in plantation area through a Presidential Instruction on a moratorium of oil palm plantation area (Presidential Instruction RI 2019). In addition, the Indonesian government is currently promoting a replanting prog. This is because most of the oil palm trees have reached the age of 25 years old. This program is very important in order to improve and increase the productivity of the FFB (fresh fruit bunch) yield. The replanting program for oil palm trees has gradually become a program of the government based on the regulation of Ministry of Agriculture No. 18 year 2016 which regulates starting from the use of high-quality seed sources and systems for replanting (Ministry of Agriculture RI 2016).

One of the reasons of low oil palm productivity, especially in smallholder plantations, is the use of low-quality seeds. Apart from being able to produce high quality oil palm seeds by crossing (generative), clonal seed could be produced through tissue culture, by means of indirect somatic embryogenesis from young leaf segments as explants. The somatic embryogenesis method indirectly through callus induction, embryo formation up to obtaining plantlets takes long time, around 3 to 3.5 years (Karyanti et al. 2019). The most important stages of somatic embryogenesis to be evaluated and observed in oil palm seed production are callus induction and embryo formation, due to these two stages had low success rate. The ability of oil palm young leaf to induce callus is still low, around 15% (Marbun et al. 2015) up to 20.48% (Karyanti et al. 2019). Aside from the callus induction stage, the embryo formation is the most important stage because it relates to the number of seeds to be produced. A low number of embryos will correlate with the low number of seeds produced. The ability of embryo formation in oil palm embryogenesis is still very low. The average ability of the embryos produced is around 3% (Marbun et al. 2015) up to 5% (Yusnita and Hapsoro 2011, Jayanthi et al. 2015, Gomes et al. 2017, Karyanti et al. 2019, Weckx et al. 2019). Not all of the embryos produced through solid culture have the ability to regenerate into plantlets. Many scientists reported that the regeneration ability of oil palm somatic embryos is around 50% (Yusnita and Hapsoro 2011, Constantin et al. 2015, Monteiro et al. 2018), and 55.56% (Karyanti et al. 2019).

In order to support the production of high-quality oil palm seeds in large number, as well as to support the availability of material for research purposes, more embryogenic callus is needed. To overcome these limitations, a method for embryogenic callus proliferation is required. One method that can be applied is a method using liquid medium by means of suspension culture. The use of liquid medium in plant propagation has been widely applied to various plants, such as sugarcane (Minarsih et al. 2013), ginseng (Kochan et al. 2019), *Bentula pendula* (Hajati et al. 2016), dates (Abohatem et al. 2017, Naik et al. 2018), Sago (Riyadi et al. 2016), *Plumbago* (Beigmohamadi et al. 2019) and oil palm (Naranjo et al. 2016, Wan Nur Syuhada et al. 2016, Naik et al. 2017, Monteiro et al. 2018). The use of liquid medium is combined with a quick immersion technique using a RITA container, shaking techniques using shaker (suspension culture) or by aeration technique in a bioreactor container. A simpler and more economical application of culture is the suspension culture method. Suspension culture has long been developed and has been widely utilized for plant propagation, secondary metabolite production (Yusuf et al. 2013, Wan Nur Syuhada et al. 2016) and providing propagules in the research activities of genetic transformation (Yarra et al. 2019) and protoplast isolation (Sallets et al. 2015).

The advantages of liquid culture upon the original solid method are that it can produce a faster proliferation. Moreover, it can increase the proliferation in large amounts of callus in a shorter period, the size of the callus will be more uniform, and less laborious (Riyadi et al. 2016). Several studies that have been conducted showed that the use of liquid media with suspension culture is more effective in the proliferation of embryogenic callus compared to quick immersion and solid culture on sago palms (Riyadi et al. 2016). Likewise, sugarcane callus using the liquid culture method was proven to increase callus biomass more than four times (Minarsih et al. 2013). Our study aimed to assess the proliferation ability of embryogenic callus derived from three different oil palm clones in repeated subcultures, and to determine the optimum subculture number for embryogenic callus proliferation in liquid medium.

MATERIALS AND METHODS

Location and time

The research was conducted at the Plant Micropropagation lab, Laboratory for Biotechnology BPPT which is located in the Puspiptek Area, Tangerang Selatan City, Banten Province. Research on embryogenic callus proliferation using liquid media had been carried out from 2018-2019.

Materials

The research materials are young leaves which are taken from three oil palm trees. The sampling locations of the three main oil palms are the oil palm collection farm Puspiptek in Banten Province (S 06° 21′ 648″, E 106° 41′ 084″, alt. 73 mamsl), Cimulang plantations (S 06° 30′ 034″, E 106° 43′ 644″, alt. 143 mamsl) and Cikasungka plantations (S 06° 33′ 637″, E 106° 32′ 642″, alt. 420 mamsl) in West Java Province. The samples taken from those 3 oil plant sources were designated as Clone-1, Clone-2, and Clone-3, respectively. All three samples were the Tenera variety. Ortets were sampled according to the SOP (Karyanti et al. 2019). Young leaf explants were sterilized by immersion method in a solution of 0.35% sodium hypochlorite for 20 minutes, furthermore in 0.45% glucose, then planted in callus induction medium (Karyanti et al. 2019), callus derived from young leaves were induced in the fourth month after planting. Observations were made to identify the appearance of embryogenic (proembryo) callus. The identified proembryos were labeled and recorded for use as material in the proliferation by suspension culture.

Liquid culture medium

The culture medium prepared was liquid media, with macro and micro salts of Murashige and Skoog (1962) as the basal medium, supplemented by thiamine hydrochloride, pyridoxine hydrochloride, nicotinic acid, glycine and 3% sucrose with the addition of 100 ppm glutamine, 1 ppm 2,4-D and 0.1 ppm NAA (Hashim et al. 2018). The pH of the medium was adjusted to 5.8 – 5.9 prior to being poured into 100 mL Erlenmeyer flasks, 40 mL each. The medium was sterilized by autoclave at 121ºC for 15 minutes.

Experimental design

This study used a completely randomized design with two factors. The first factor was different types of clones and the second factor was subculture. Each treatment was repeated 3 times, and observations were made every 2 weeks. Subcultures were performed every 8 weeks.

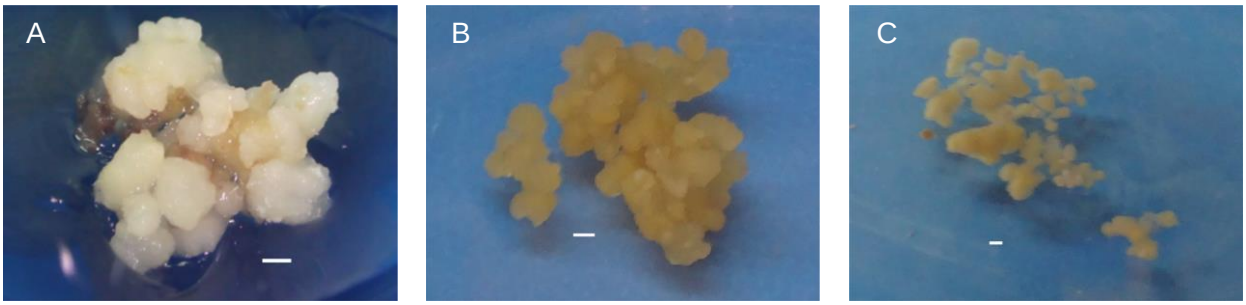

Figure 1. Embryogenic callus (A), callus mass (B), and embryogenic callus in liquid medium (C). (White bar: 1 mm scale)

Embryogenic callus planting and incubation

The prepared embryogenic or proembryo callus (Figure 1A) was carefully weighed using sterile Petri disk at about 0.1 - 0.2 g (Figure 1B). Subsequently, the embryogenic callus was cultured into the liquid medium slowly and the lid of flask was closed tightly to ensure the culture remained sterile (Figure 1C). All embryogenic calluses that had been induced in liquid medium were placed on an incubator shaker at a speed of 70–90 rpm at 27 ºC without light.

Callus proliferation in liquid medium was incubated for 8 weeks. Subculture of the embryogenic callus into fresh liquid medium was repeated for 7 times in eight weeks intervals. In addition, 0.1–0.2 g of embryogenic callus was planted in the regeneration media in every subculture. Callus was incubated indoors with the white fluorescent light of 1,500 Lux at an ambient temperature of 26–27 ºC.

Observation and data analysis

Observations were made visually by identifying the development of callus proliferation at week four and eight. In the eighth week after planting, qualitative and quantitative data were collected on callus weight, callus color and callus structure. The observation of regeneration ability was done visually every two weeks and documentation data was taken at the eight weeks after planting. Callus fresh weight data were analyzed using the SPPS (Statistical Package for the Social Sciences) program with the Duncan test (DMRT) at the 0.05 level. Observations were made in each subculture and then the analyzing data of each subculture could be compared.

RESULTS AND DISCUSSION

Callus proliferation and development

The increase in weight of embryogenic callus was significantly different among Clone-1, Clone-2 and Clone-3. The average increased in weight of embryogenic callus of Clone-2 at the eighth week of culture was the highest, i.e. 1.039 g, compared to those of Clone-1 and Clone-3 which were 0.651 and 0.557 g, respectively (Figure 2). These results indicated that oil palm callus embryogenic proliferation in this experiment was significantly affected by genotypes of the ortet.

Embryogenic callus was multiplied continuously by means of subculture. The weight of the embryogenic callus was increased in line with the number of subcultures, up to the fourth subculture. The increase in callus weight was significantly different in the 1st, 2nd and 3rd subcultures, but not significantly different between the 3rd and 4th subcultures (Figure 3). After the fourth subculture, the increasing number of subcultures reduced the callus weight significantly, especially in the 5th, 6th and 7th subcultures (Figure 3). These results showed that the proliferation of callus cells has a limit, which increase to a maximum extent and decrease afterward. This result implied that subculturing of the oil palm embryogenic callus for multiplication is restricted to the 3rd or 4th subcultures only.

Decreasing rate of callus weight may occur due to the development of embryogenic callus into somatic embryos, so that the energy in the callus was used for developing into somatic embryos (Guillou et al. 2018). However, in our experiment, it is supposed that the decline in proliferation ability was due to the callus being saturated and aged, so that its proliferation ability decreases along with the increasing number of subcultures.

The evaluation of callus weight for each clone is shown in Figure 4. In all of the oil palm clones, callus weight increased gradually in each subculture. The clone had a different optimal increase in callus weight to each other. In Figure 4, it can be seen that the average highest increase in callus weight in Clone-1 occurred in subculture 3, whereas in Clone-2 occurred in subculture 4 and Clone-3 occurred

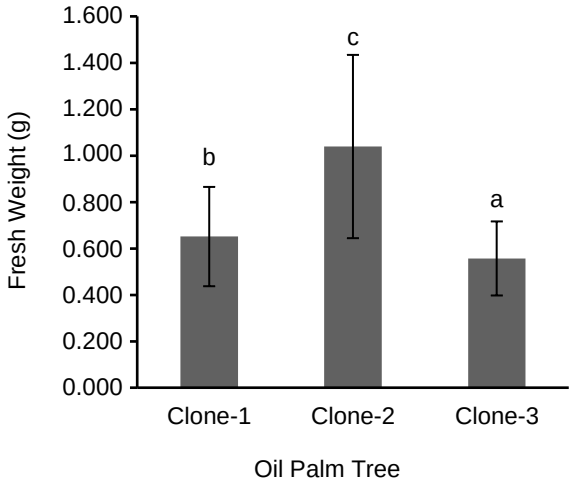

Figure 2. Increase in callus weight of oil palm clones

in subculture 3. After the 3rd and 4th subcultures the callus weight started to decrease. The mechanism for increasing the callus weight was different to each subculture and each clone, indicating that the callus of each clone responded differently to the proliferation medium. It was revealed that the Clone-1 had a fast and high increased in callus weight, obtaining an average increased of callus weight in the 1st subculture at 0.73 g and continued to increase until the 4th subculture at 1.72 g. After the 4th culture, the average weight of callus subculture, the average weight of callus decreased steadily, and an average of callus weight kept decreasing at the 7th subculture, which were 0.38 to 0.57 g.

The proliferation ability of each clone was not the same, some had fast proliferation abilities and some were slow. Each clone had a different ability to produce embryogenic callus. The proliferation ability in suspension culture was influenced by the quality of the embryogenic callus used. Clones that induced the initial callus crumb and yellowish-white solid can produce potential embryogenic callus. An important factor affecting the clones' ability to produce potential callus may be due to the clone genotype and maintenance factor during cultivation. So, it is important to carefully select the mother tree to support the production of embryogenic callus.

The highest proliferative ability of Clone-1 in the 4th subculture was 7.38× of initial callus weight. The highest increase of Clone-2 was in the 4th subculture, i.e. 17.68×, while the highest increase of Clone-3 was in the 3rd subculture, i.e. 7.29× (Figure 5 and 6). These

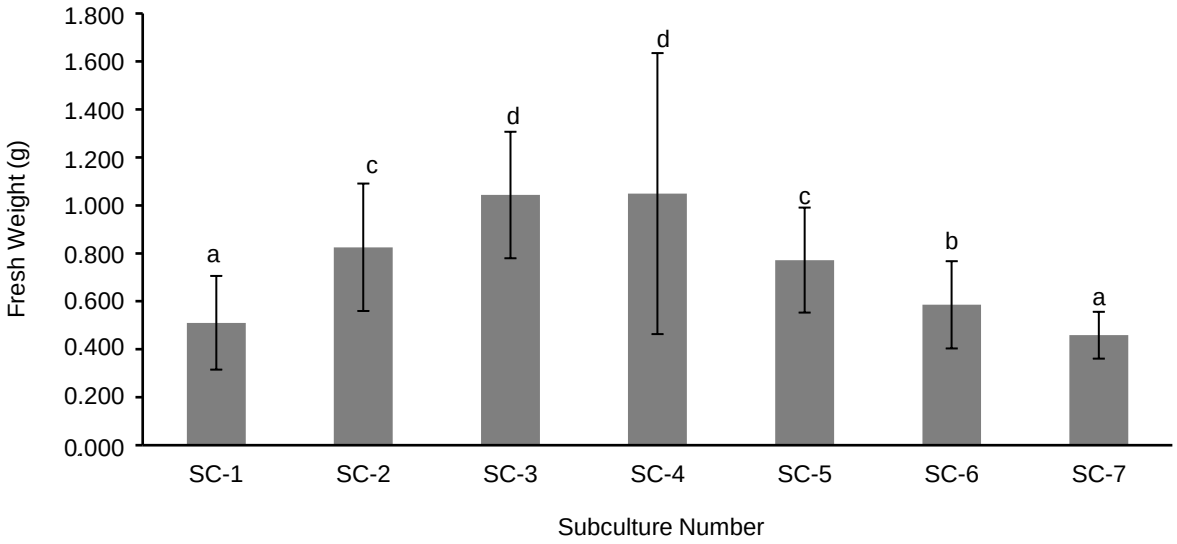

Figure 3. Average weight of oil palm embryogenic callus per subculture. The abbreviations SC-1 to SC-7 represent 1st subculture to 7th subculture, respectively

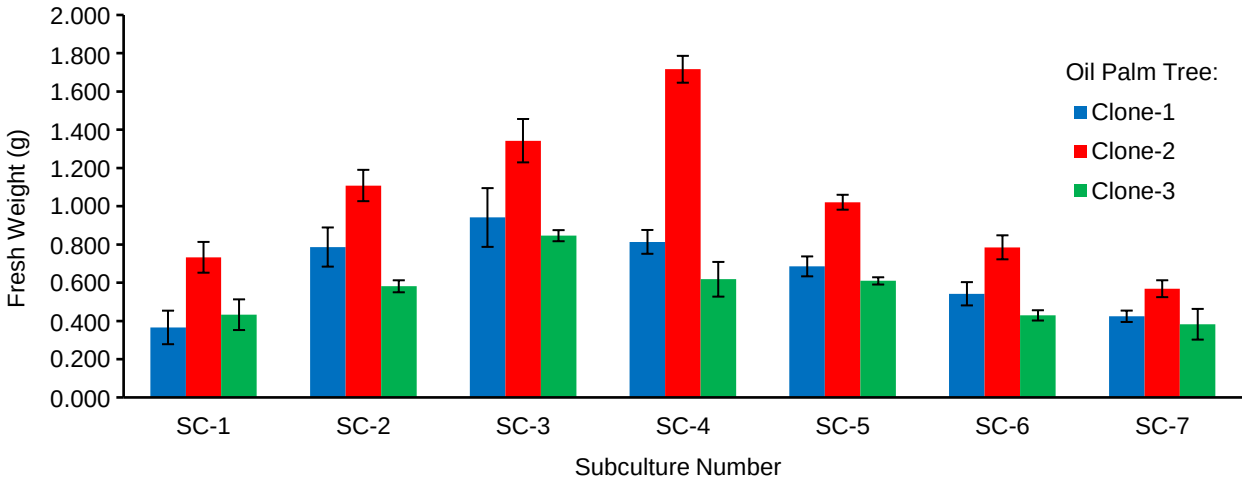

Figure 4. Increased weight of embryogenic callus in all oil palm clones. The abbreviations SC-1 to SC-7 represent 1st subculture to 7th subculture, respectively

results indicated that the Clone-2 has a higher proliferation ability compared to the other two clones. The ability of callus proliferation of Clone-2 possibly was influenced by the quality of the callus induced and the initial endogenous hormone that contained in the palm leaves. So, it is important that in the initial stages of callus and embryo induction one should use the right culture media in order to produce embryogenic callus of high quality.

Figure 6 is a visual observation by means of photo documentations. The results showed that the proliferation of each clone was differing. In the 1st subculture at the eighth week after planting, it was seen that the embryogenic callus proliferation of Clone-2 covered all surfaces area compared to the other clones. The same phenomenon was observed in the following subcultures. The embryogenic callus proliferation of Clone-2 looks the same to the 1st, 2nd, 3rd and 4th subcultures and embryogenic callus accumulation occurs as subcultures number increased. Figure 6 shows that each clone has different proliferation abilities.

Color and structure of embryogenic callus

Oil palm embryogenic callus exhibited color that were all the same at all subculture stages, which was cream in color (Table 1). The color of the embryogenic callus on MS medium for all clones and from 1st up to 7th subculture did not change, it remained cream in color (Figure 7). The color of the callus probably was influenced by the nutrient and the type of growth regulator added into medium. There was no brown callus found during the experiment. It might indicate that the MS medium, supplemented by vitamins, the types of growth regulators 2,4-D and NAA and their concentrations were suitable for oil palm suspension cultures.

In addition to callus color, structure of callus was also observed. The embryogenic callus produced by each clone in each subculture resulted in different structures (Figure 7 and Table 1). The callus structure of Clone-2 generated dry and friable embryogenic callus in every subculture. Different phenomenon was observed in clone 1. The callus structure from the 1st up to 3rd subcultures resulted in dry and friable callus structure, however at the 4th up to 7th subcultures it produced slimy callus structure. Likewise, in Clone-3, the dry and friable callus structure was detected in the 1st subculture, and in subsequent subculture up to 7th subcultures, the wet and soft callus structure was observed.

The resulting callus structures derived from the three clones were dried friable and slimy or softened and watery. These results indicated that several embryogenic callus from different clones on MS medium produced different responses. It can be seen on Clone-2, that all of the callus structures in every subculture was dried friable. Conversely, at the other two clones, the callus structures were initially dried friable, and subsequently became watery callus. This is presumably because the ability of the cells in the callus derived from each clone has different responses to the liquid medium used. In addition, it is also likely that there was an effect of the types of growth regulators added that softened the cells of callus.

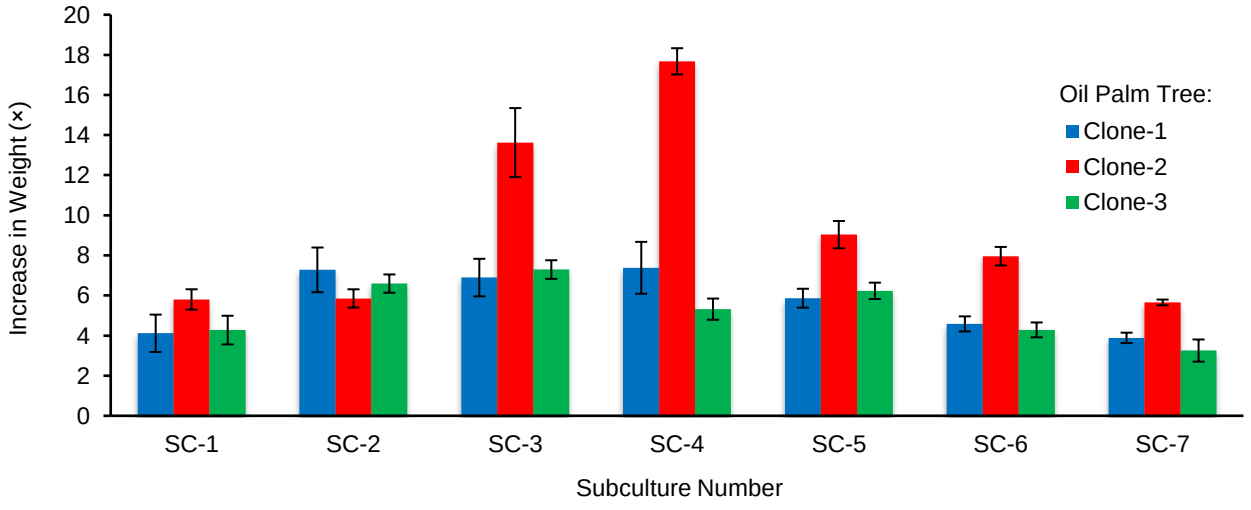

Figure 5. Ability of embryogenic callus proliferation of all oil palm clones. The abbreviations SC-1 to SC-7 represent 1st subculture to 7th subculture, respectively

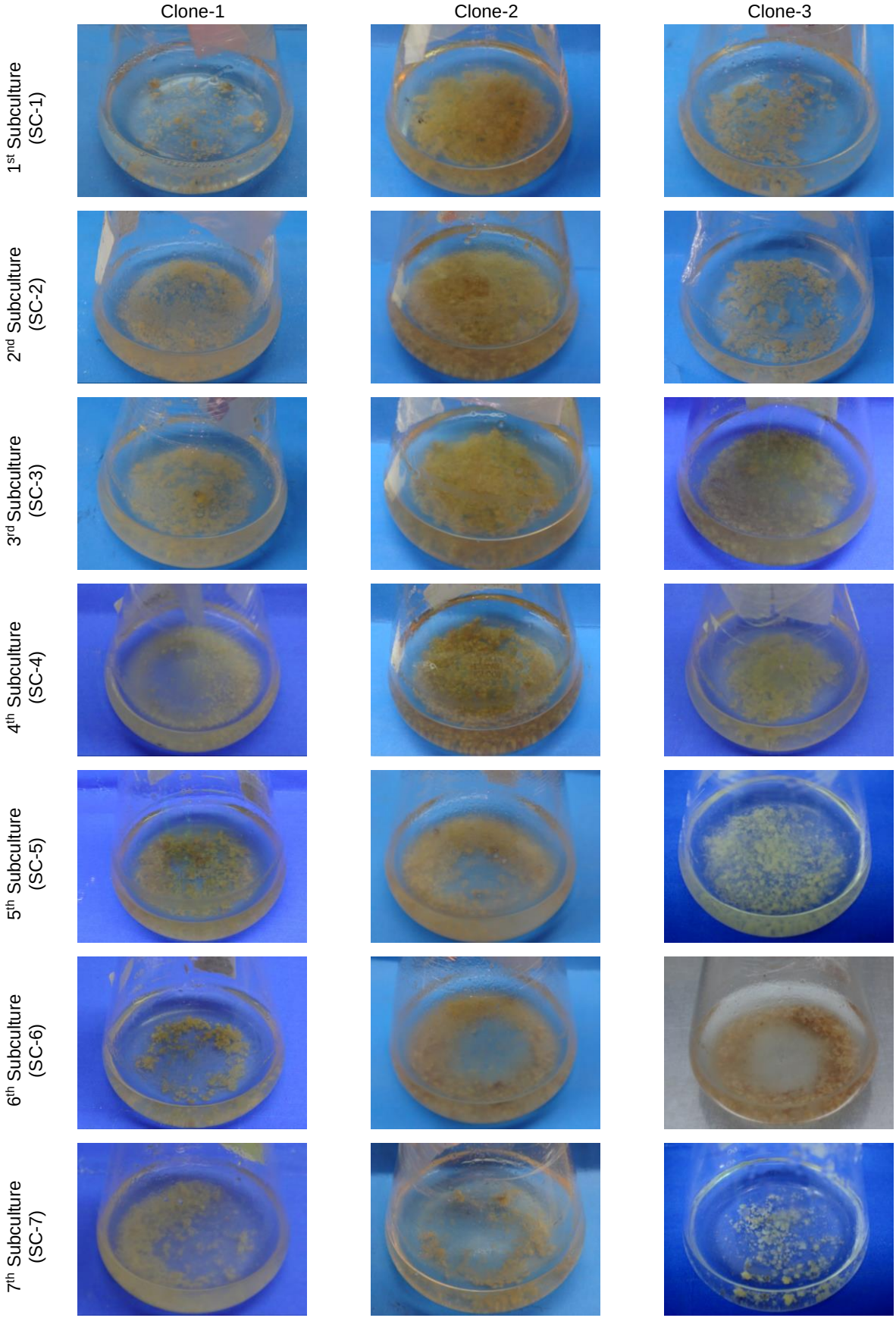

Figure 6. The proliferation of oil palm embryogenic callus from three oil palm genotypes from the 1st up to 7th subcultures in Erlenmeyer flasks (100 mL volume, 6 cm base diameter)

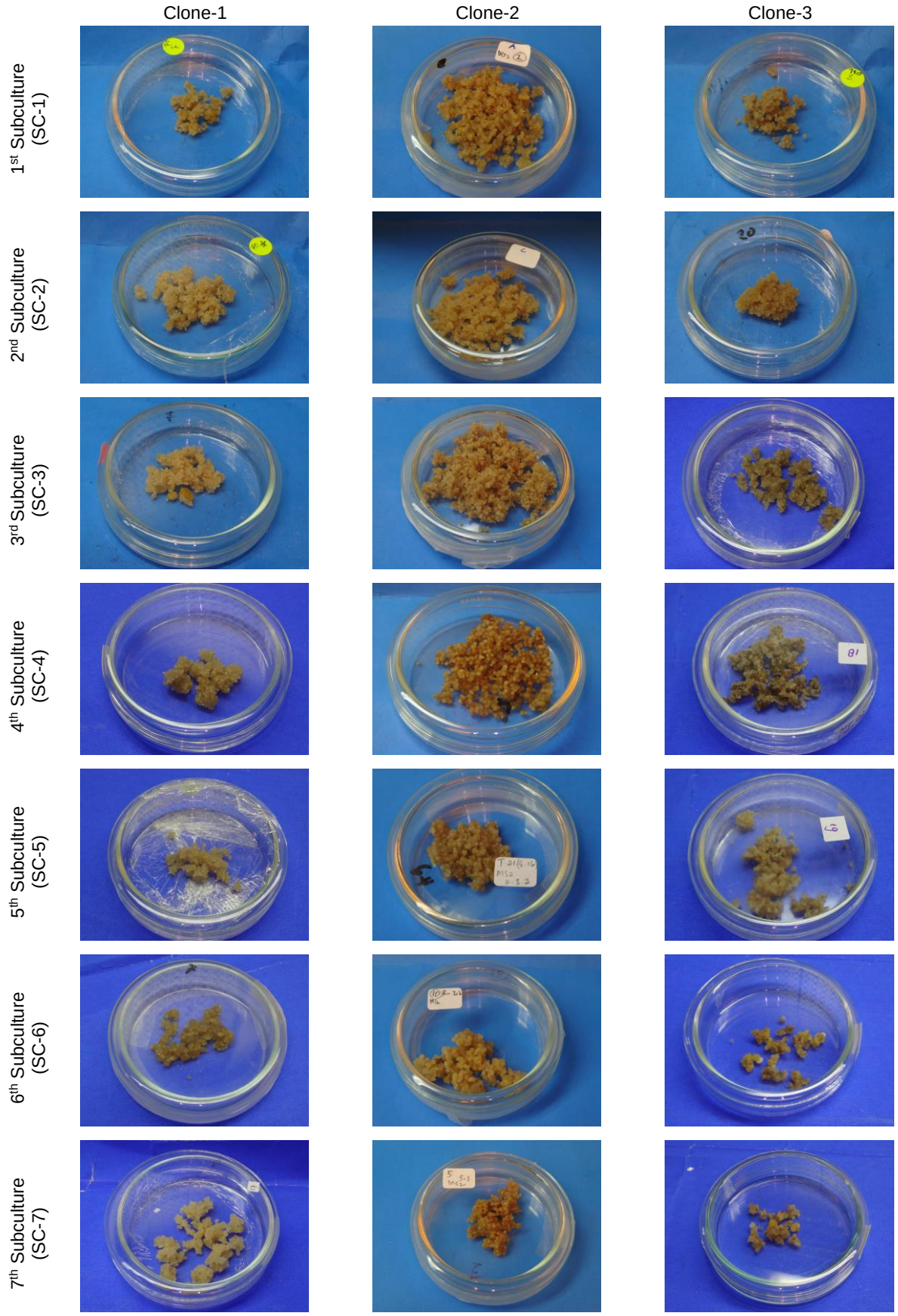

Figure 7. Color and structure of embryogenic callus clones in the 1st up to 7th subcultures in petri dishes (5 cm base diameter)

Table 1. Color and structure of oil palm embryogenic callus in 1st subculture (SC-1) to 7th subculture (SC-7)

Treatment		Color	Structure
Samples	Subculture No		
Clone-1	SC-1	Cream	Friable
	SC-2	Cream	Friable
	SC-3	Cream	Friable
	SC-4	Cream	Wet and soft
	SC-5	Cream	Wet and soft
	SC-6	Cream	Wet and soft
	SC-7	Cream	Wet and soft
Clone-2	SC-1	Cream	Friable
	SC-2	Cream	Friable
	SC-3	Cream	Friable
	SC-4	Cream	Friable
	SC-5	Cream	Friable
	SC-6	Cream	Friable
	SC-7	Cream	Friable
Clone-3	SC-1	Cream	Friable
	SC-2	Cream	Wet and soft
	SC-3	Cream	Wet and soft
	SC-4	Cream	Wet and soft
	SC-5	Cream	Wet and soft
	SC-6	Cream	Wet and soft
	SC-7	Cream	Wet and soft

Table 2**.** Percentage of regeneration ability of embryogenic callus clones in 1st subculture (SC-1) up to 7th subculture (SC-7)

Treatment		Regeneration (%) after 10 MST
Samples	Subculture No	
Clone-1	SC-1	100
	SC-2	100
	SC-3	100
	SC-4	100
	SC-5	100
	SC-6	100
	SC-7	100
Clone-2	SC-1	100
	SC-2	100
	SC-3	100
	SC-4	100
	SC-5	100
	SC-6	100
	SC-7	100
Clone-3	SC-1	100
	SC-2	100
	SC-3	100
	SC-4	80
	SC-5	80
	SC-6	80
	SC-7	80

Regeneration ability

The embryogenic callus propagated in suspension culture must be able to regenerate into somatic embryos, and then develop into new plants. All callus proliferated from the 1st up to 7th subculture of all clones were planted in the regeneration medium. Data from visual observation were summarized in Table 2, which showed that the percentage of oil palm callus cultures that were able to regenerate into embryo somatic. The results revealed that not all embryogenic calluses could regenerate 100% into shoots. In Clone-3, from subcultures 4 up to 7, it was found that only 80% of the oil palm embryogenic callus could regenerate into shoots. Whereas in Clone-1 and Clone-2, it was found that 100% callus from all subcultures, regenerated into shoots (Table 2).

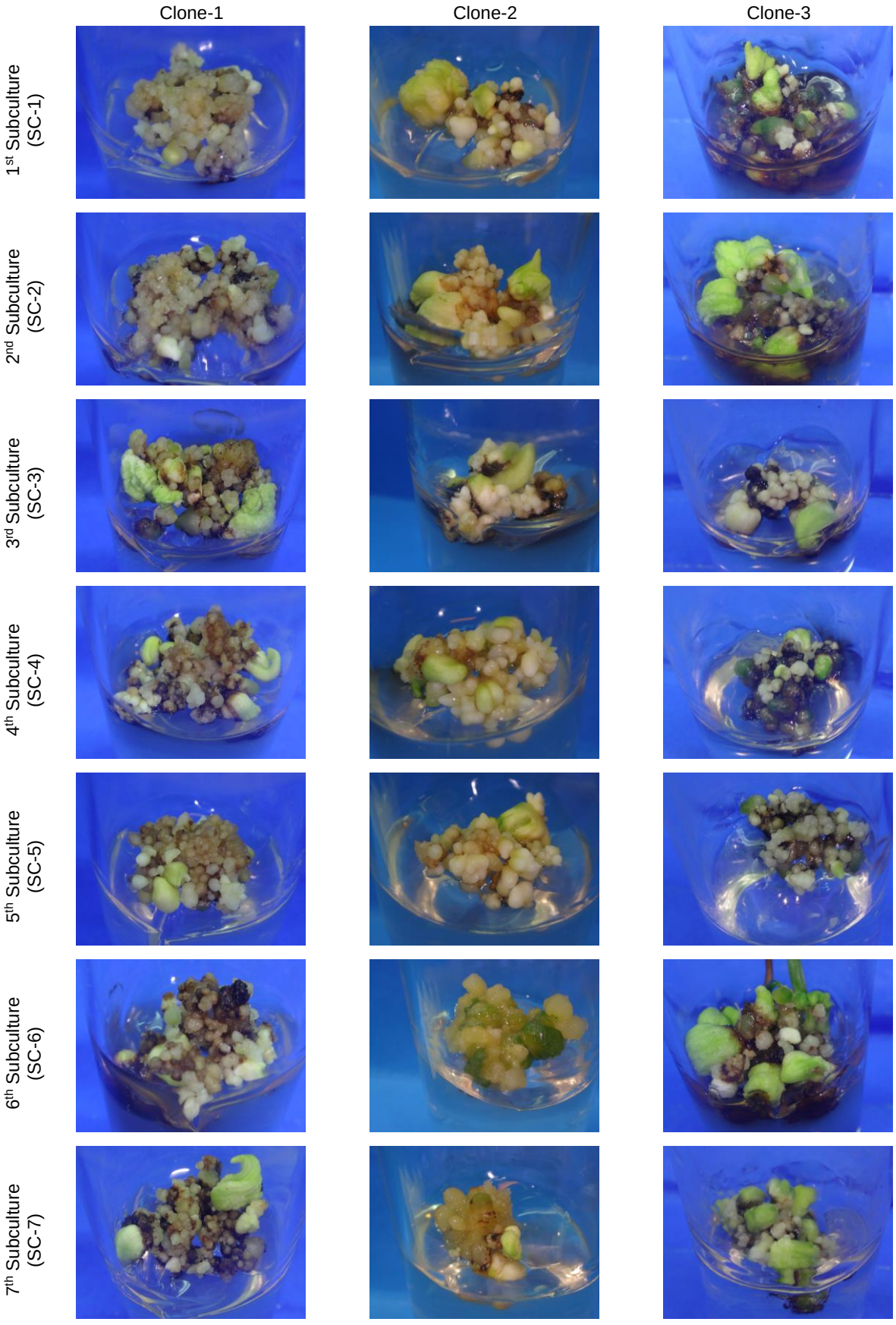

Figure 8. Prospective new plantlets derived from all clones of callus multiplication resulted from 1st up to 7th subcultures in glass tubes (5 cm diameter)

The ability of cells of oil palm callus to generate into plantlets after sub culturing was differing for each clone. Genotype and structure of callus were believed to affect the ability of cells to regenerate after repeated subcultures. There were genotypes in which callus cells were able to survive and regenerate into further stages of morphogenesis regardless of repeated subcultures. On the other hand, there were also genotypes in which callus cells became damaged or died. Figure 8 is a representative of embryogenic callus from each clone in each subculture grown in the regeneration medium, and the response showed the signs of regeneration. It appears that all of the calli have the potential to regenerate and produce prospective shoots.

Decreasing percentage of regeneration ability of Clone-3 callus from the 4th up to 7th subcultures was believed due to the slimy callus structure, and some of the cells were not embryogenic anymore. These results also indicated that most of the embryogenic callus produced up to the 7th subculture had good cell quality so that they could adapt to the regeneration medium to produce new shoots.

CONCLUSION

The proliferation of oil palm embryogenic callus using suspension culture was strongly influenced by genotype. Depending on the genotype, the proliferation ability of embryogenic callus was increase 7 times compared to initial weight. The fresh weight of embryogenic callus was increased from the 1st up to 4th subcultures and then decreased in the 5th to 7th subcultures. The combination of liquid media used had no effect on the callus color, nevertheless it had an effect on the callus structure. The ability of embryogenic callus to regenerate was influenced by the structure of the callus. Dry and friable callus had the potential to regenerate at 100%, whereas the softened and watery callus was regenerated at 80%.

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REFERENCES

Abohatem MA, Bakil Y, Baaziz M (2017) Plant regeneration from somatic embryogenic suspension cultures of date palm, In Al-Khayri JM, Jain SM, Johnson DV (Eds), Date Palm Biotechnology Protocols: Tissue Culture Applications. Humana Press, New York, pp.203-214. doi: 10.1007/978-1-4939-7156-5

Beigmohamadi M, Movafeghi A, Sharafi A, Jafari S, Danafar H (2019) Cell suspension culture of *Plumbago europaea* L. towards production of plumbagin. Iran J Biotechnol 17: e2169. doi: 10.21859/ijb.2169

Constantin M, Nchu WA, Godswill NN, Wiendi NMA, Wachjar A, Frank NEG (2015) Induction of oil palm (*Elaeis guineensis* Jacq. var. Tenera) callogenesis and somatic embryogenesis from young leaf explants. J Appl Biol Biotechnol 3: 004-010. doi: 10.7324/JABB.2015.3402

Directorate General of Estate Crops (2019) Tree crop estate statistics of Indonesia 2018-2020: Palm oil. Secretariate of Directorate General of Estate Crops. Ministry of Agriculture Republic Indonesia, Jakarta

Gomes HT, Bartos PMC, Scherwinski-Pereira JE (2017) Dynamics of morphological and anatomical changes in leaf tissues of an interspecific hybrid of oil palm during acquisition and development of somatic embryogenesis. Plant Cell Tiss Organ Cult 131: 269-282. doi: 10.1007/s11240-017-1282-8

Guillou C, Fillodeau A, Brulard E, Breton D, De Faria Maraschin S, Verdier D, Simon M, Ducos JP (2018) Indirect somatic embryogenesis of *Theobroma cacao* L. in liquid medium and improvement of embryo-to-plantlet conversion rate. In Vitro Cell Dev Biol Plant 54: 377-391. doi: 10.1007/s11627-018-9909-y

Hajati RJ, Payamnoor V, Bezdi KG, Chashmi NA (2016) Optimization of callus induction and cell suspension culture of *Betula pendula* Roth for improved production of betulin, betulinic acid, and

antioxidant activity. In Vitro Cell Dev.Biol Plant 52: 400-407. doi: 10.1007/s11627-016-9773-6

Hashim AT, Ishak Z, Rosli SK, Ong-Abdullah M, Siew-Eng O, Husri MN, Bakar DA (2018) Oil Palm (*Elaeis guineensis* Jacq.) Somatic Embryogenesis. In: Jain S, Gupta P (Eds.) Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants. Forestry Science vol 85. Springer. doi: 10.1007/978-3-319-79087-9_18

Jayanthi M, Susanthi B, Mohan NM, Mandal PK (2015) *In vitro* somatic embryogenesis and plantlet regeneration from immature male inflorescence of adult dura and tenera palms of *Elaeis guineensis* (Jacq.). Springerplus 4: 256. doi: 10.1186/s40064-015-1025-4

Karyanti, Rismayanti, Riyadi A, Herliana L, Khairiyah H, Sukarnih T, Rudiyana Y, Setia WW, Rochandi A, Sarman, Sarifudin D (2019) Clonal propagation of oil palm (*Elaeis guineensis* Jacq.) seeds. Final Report of Collaboration Research with Astra Agro Lestari. Laboratory for Biotechnology, Agency for the Assessment and Application of Technology, Tangerang Selatan

Kochan E, Szymanska G, Grzegorczy-Karolak I, Szymczyk P, Sienkiewicz M (2019) Ginsenoside and phenolic compounds in hydromethanolic extracts of American ginseng cell cultures and their antioxidant properties. Acta Soc Bot Pol 88: 3638. doi: 10.5586/asbp.3638

Marbun CLM, Toruan-Marhius N, Reflini, Utomo C, Liwang T (2015) Micropropagation of embryogenic callus of oil palm (*Elaeis guineensis* Jacq.) using temporary immersion system. Procedia Chem 14: 122-129. doi: 10.1016/j.proche.2015.03.018

Minarsih H, Riyadi I, Sumaryono, Budiani A (2013) Micropropagation of sugarcane (*Saccharum officinarum* L.) using temporary immersion system. Menara Perkebunan 81: 1-8. doi: 10.22302/iribb.jur.mp.v81i1.53

Ministry of Agriculture RI (2016) Replanting guideline for oil palm plantation. Regulation of the Ministry of Agriculture No. 18/ permentan/KB.330/5/2016. Ministry of Agriculture of the Republic of Indonesia, Jakarta

Ministry of Agriculture RI (2019) regarding Stipulation of Indonesia's Oil Palm Area Coverage. Decision of the Minister of Agriculture of the Republic of Indonesia No. 833/KPTS/SR.020/M/12/2019. Ministry of Agriculture of the Republic of Indonesia, Jakarta

Monteiro TR, Freitas EO, Nogueira GF, Scherwinski-Pereira JE (2018) Assessing the influence of subcultures and liquid medium during somatic embryogenesis and plant regeneration in oil palm (*Elaeis guineensis* Jacq.). J Hortic Sci Biotechnol 93: 196-203. doi: 10.1080/14620316.2017.1360156

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco cultures. Phys Plant 15(3): 473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x

Naik PM, Al-Khayri JM (2017) Extraction and estimation of secondary metabolites from date palm cell suspension cultures. Methods Mol Biol 1637: 319-332. doi: 10.1007/978-1-4939-7156-5_26

Naik PM, Al-Khayri JM (2018) Cell suspension culture as a means to produce polyphenols from date palm (*Phoenix dactylifera* L.). Cienc Agrotec 42: 464-473. doi: 10.1590/1413-70542018425021118

Naranjo EJ, Fernandez Betin O, Urrea Trujillo AI, Callejas Posada R, Atehortua Garces L (2016) Effect of genotype on the *in vitro* regeneration of *Stevia rebaudiana* via somatic embryogenesis. Acta Biol Colomb 21: 87-98. doi: 10.15446/abc.v21n1.47382

Presidential Instruction RI (2019) Termination of granting of new permits and improving management of primary natural forests and peatlands. Presidential Instruction of the Republik of Indonesia No. 5 Tahun 2019. Ministry of State Secretariat of the Republic of Indonesia, Jakarta

Riyadi I, Efendi D, Purwoko BS, Santoso D (2016) Indirect somatic embryogenesis in sago palm (*Metroxylon sago* Rottb.) using suspension culture and temporary immersion solid media system. J AgroBiogen 12: 37-44. doi: 10.21082/jbio.v12n1.2016.p37-44

Sallets A, Delimoy A, Boutry M (2015) Stable and transient transformation of *Artemisia annua* suspension cells. Plant Cell Tiss Organ Cult 120: 797-801. doi: 10.1007/s11240-014-0631-0

Wan Nur Syuhada WS, Rasid OA, Parveez GKA (2016) Evaluation on the effects of culture medium on regeneration of oil palm plantlets from immature embryos (IE). J Oil Palm Res 28: 234-239. doi: 10.21894/jopr.2016.2802.12

Weckx S, Inze D, Maene L (2019) Tissue culture of oil palm: Finding the balance between mass propagation and somaclonal variation. Front Plant Sci 10: 722. doi: 10.3389/fpls.2019.00722

Yarra R, Jin L, Zhao Z, Cao H (2019) Progress in tissue culture and genetic transformation of oil palm: An overview. Int J Mol Sci 20: 5353. doi: 10.3390/ijms20215353

Yusnita, Hapsoro D (2011) *In Vitro* callus induction and embryogenesis of oil palm (*Elaeis guineensis* Jacq.) from leaf explants. Hayati J Biosci 18: 61-65. doi: 10.4308/hjb.18.2.61

Yusuf NA, Annuar MSM, Khalid N (2013) Physical stress for overproduction of biomass and flavonoids in cell suspension cultures of *Boesenbergia rotunda*, Acta Physiol Plant 35: 1713-1719. doi: 10.1007/s11738-012-1178-5