

## PRIMARY AND SECONDARY SOMATIC EMBRYOGENESIS OF CACAO: THE EFFECT OF EXPLANT TYPES AND PLANT GROWTH REGULATORS

### *Embriogenesis Somatik Primer dan Sekunder pada Kakao: Pengaruh Jenis Eksplan dan Zat Pengatur Tumbuh*

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#### ABSTRACT

The success of cacao somatic embryogenesis is affected by many factors, including the basal salt medium, the genotype, the explant type, and the concentration and composition of plant growth regulators (PGRs). The study aimed to evaluate the effects of PGRs composition on the primary somatic embryo (PSE) response and the effect of explant type and PGRs composition used in inducing PSE on the secondary somatic embryogenesis (SSE) response. PSEs were induced from basal petal and staminoid explants of MCC 01 and MCC 02 clones on DKW medium containing 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.5 mg l<sup>-1</sup> or 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.125 – 0.250 mg l<sup>-1</sup> + thidiazuron (TDZ) 2.5 – 5 µg l<sup>-1</sup> or 2,4-D 2 mg l<sup>-1</sup> + TDZ 10 µg l<sup>-1</sup>. Genotype, explant type, and PGR composition dependently affected PSE response. The best PSE response was obtained from staminoid explant of MCC 02 clone on medium containing 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.5 mg l<sup>-1</sup> (20%, 9 embryos). The explant type and PGR composition used in inducing PSEs affect the SSE response. The highest SSE response of MCC 01 clone was obtained from petal explant with medium containing 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.5 mg l<sup>-1</sup>. The formation of SSEs could increase the multiplication rate of MCC 01 clone by 7 times.

[**Keywords:** *explants, PGRs, primary somatic embryogenesis, secondary somatic embryogenesis, Theobroma cacao L.*]

#### ABSTRAK

Keberhasilan embriogenesis somatik pada tanaman kakao dipengaruhi oleh banyak faktor, di antaranya komposisi media dasar, genotipe, jenis eksplan, serta komposisi dan konsentrasi zat pengatur tumbuh (ZPT). Penelitian bertujuan untuk mengevaluasi pengaruh komposisi ZPT terhadap embriogenesis somatik primer kakao serta pengaruh jenis eksplan dan komposisi ZPT yang digunakan untuk menginduksi embriogenesis somatik primer terhadap pembentukan embrio somatik sekunder. Embriogenesis somatik primer diinduksi dari eksplan mahkota bunga dan staminoid klon kakao MCC 01 dan MCC 02 pada media DKW dengan penambahan 2,4-D 2 mg l<sup>-1</sup> + kinetin 0,5 mg l<sup>-1</sup> atau 2,4-D 2 mg l<sup>-1</sup> + kinetin 0,125 – 0,250 mg l<sup>-1</sup> + thidiazuron

(TDZ) 2,5 – 5 µg l<sup>-1</sup> atau 2,4-D 2 mg l<sup>-1</sup> + TDZ 10 µg l<sup>-1</sup>. Respons pembentukan embrio somatik primer terbaik diperoleh dari eksplan staminoid klon MCC 02 pada media yang mengandung 2,4-D 2 mg l<sup>-1</sup> + kinetin 0,5 mg l<sup>-1</sup> (20%, 9 embrio). Jenis eksplan dan komposisi ZPT yang digunakan untuk menginduksi pembentukan embrio somatik primer berpengaruh terhadap respons embriogenesis somatik sekunder. Respons embriogenesis somatik sekunder paling baik pada klon MCC 01 diperoleh dari eksplan mahkota bunga dengan media yang mengandung 2,4-D 2 mg l<sup>-1</sup> + kinetin 0,5 mg l<sup>-1</sup>. Pembentukan embrio somatik sekunder dapat meningkatkan laju multiplikasi klon MCC 01 sebesar 7 kali.

[**Kata kunci:** *eksplan, embriogenesis somatik primer, embriogenesis somatik sekunder, zat pengatur tumbuh, Theobroma cacao L.*]

#### INTRODUCTION

Developing a regeneration system of cacao through somatic embryogenesis is important for various purposes, including plant propagation, genetic improvement, germplasm conservation, and genetic transformation. The system has been developed at least ten laboratories in the world with various explant types, media, and plant growth regulators (PGRs). Basal petal and staminoid are two types of explant commonly used for cacao somatic embryogenesis, but the responses of those explants are different depending on the genotype, as well as the response of the genotype (Ajijah 2014; Ajijah et al. 2016; Ajijah and Hartati 2016; Ajijah et al. 2016; Garcia et al. 2016). The success of cacao somatic embryogenesis is also affected by the basal salt medium (Ajijah 2016) and the concentration and composition of PGRs (Issali et al. 2008; Ajijah 2016; Ajijah et al. 2016; Garcia et al. 2016).

The best medium for producing cacao somatic embryos is DKW medium (Ajijah 2016; Li et al. 1998)

with the addition of 2,4-D combined with several types of cytokinin including thidiazuron (TDZ) (Li et al. 1998; Buah 2010; Ajijah et al. 2014), 2ip (Tan and Furtek 2004), kinetin (Ajijah et al. 2016) or adenine (Avivi et al. 2010). Most of the Indonesian cacao clones have good responses to kinetin (Ajijah et al. 2016), nevertheless, some genotypes still have a low frequency of somatic embryogenesis (Ajijah and Hartati 2016) as well as their responses to TDZ (Li et al. 1998; Ajijah et al. 2014). It is not known yet whether kinetin and TDZ could synergize to increase the success of cacao somatic embryogenesis compared to kinetin and TDZ singly.

Secondary somatic embryogenesis (SSE) is the phenomenon whereby new somatic embryos are initiated or induced from primary somatic embryos (PSEs) (Yang et al. 2013). According to Pinto et al. (2008), in some species, PSE is less efficient compared to SSE. SSE is reported could increase multiplication rate and regeneration efficiency in many plant species, especially those who have low PSE responses (Li et al. 2002). Inducing SSEs from PSEs in cacao has been reported by Maximova et al. (2002) and Tan and Furtek (2004) with varied success rates depending on the genotype and PGRs composition. Other studies also reported factors affecting SSE formation in many plant species, such as basal salt medium, PGRs, and light in *Eucalyptus globulus* L. (Pinto et al. 2008); medium phases in *Hovenia dulcis* Thunb. (Yang et al. 2013); basal salt medium and sucrose concentration in chrysanthemum (Htay et al. 2013); medium phases and PGRs in *Coffea arabica* var. Catimor (Silva et al. 2005); PGRs and carbon sources in carnation (Karami and Deljou 2008). However, those studies only focused on evaluating factors affecting SSE without evaluating whether culture conditions used for producing PSEs, such as explant types, PGRs, and another medium components, will affect the formation of SSEs. So far, there is no information yet, whether explant types and PGRs composition used in inducing callus for PSE production affect the SSE formation, especially in cacao. Therefore, this study aimed to evaluate the effects of PGRs composition on the PSE formation and the effect of explant types and PGRs composition used in inducing PSE on the SSE formation.

## MATERIALS AND METHODS

### Plant Materials and Surface Sterilization

The research was conducted at the Tissue Culture Laboratory of the Indonesian Center for Estate Crops Research and Development, Bogor, West Java, and the Tissue Culture Laboratory of Indonesian Industry

and Freshener Crops Research Institute, Sukabumi, West Java. Staminoïd and basal petals from the flower bud of MCC 01 and MCC 02 cacao clones were used as explants. Flower buds were taken from Sub-Station Cacao Research Gardens belonging to the South Sulawesi Provincial Government at Lebojaya village, Konda district, South Konawe regency.

The flower buds were placed in glass bottles containing sterilized cold distilled water and then stored in the refrigerator until they were planted. Before planting, the flower buds were sterilized by soaking and shaking in 5% Clorox for 10 minutes then rinsed with sterilized distilled water three times each for 10, 5, and 5 minutes, respectively. Petals and staminoids were extracted from flower buds using sterile scalpels in a sterile petri dish.

### Effect of PGRs on Primary Somatic Embryogenesis

The first step of cacao primary somatic embryogenesis process is callus induction. Callus was induced from staminoid and basal petal explants of MCC 01 and MCC 02 clones on primary callus induction medium containing DKW basal salt medium with the addition of five PGRs combinations (Table 1). After two weeks, callus was transferred into the secondary callus growth medium consisting of WPM basal salt medium with addition of 2,4-D 2 mg l<sup>-1</sup> and kinetin 0.125 mg l<sup>-1</sup> (Ajijah et al. 2016). The cultures were incubated at 25 °C in dark conditions.

After two weeks on a secondary callus growth medium, callus was transferred onto DKW medium without PGR for inducing the formation and maturation of PSEs. Cultures were incubated at 25 °C in dark conditions and subcultured on the same medium every 3 weeks until mature cotyledonary stage of PSEs were obtained. Tests were carried out on the effect of explant types (basal petals and staminoids) and PGRs compositions (Table 1) on the percentage of PSE formations and the number of PSEs per responsive explant. Each treatment consisted of five replications, and each experimental unit consisted of 10 explants.

**Table 1. Plant growth regulators (PGRs) composition in primary callus induction medium.**

Medium	PGRs composition		
	2,4-D (mg l <sup>-1</sup> )	Kinetin (mg l <sup>-1</sup> )	Thidiazuron (µg l <sup>-1</sup> )
M1	2	0.5	
M2	2	0.125	2.5
M3	2	0.125	5.0
M4	2	0.250	5.0
M5	2		10.0

### Effect of Explant Types and PGRs on Secondary Somatic Embryogenesis

The PSEs from the first experiment were used as explants for producing SSEs in the second experiment. The cotyledonary stage of the PSEs was cultured on the DKW medium containing adenine and amino acids and subcultured into the same medium every month until the SSEs were formed. The cotyledonary stage of PSEs of MCC 01 clone originated from petal and staminoïd-derived callus induced using a medium containing 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.25 \text{ mg l}^{-1}$  + TDZ  $5.0 \text{ } \mu\text{g l}^{-1}$  (M4) were used as explants to evaluate the effect of explant types on the SSE formation. While the cotyledonary stage of PSEs of MCC 01 clone originated from petal-derived callus induced using medium containing 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.5 \text{ mg l}^{-1}$  (M1), 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.125 \text{ mg l}^{-1}$  + TDZ  $2.5 \text{ } \mu\text{g l}^{-1}$  (M2), or 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.25 \text{ mg l}^{-1}$  + TDZ  $5.0 \text{ } \mu\text{g l}^{-1}$  (M4) were used as explants to evaluate the effect of PGRs on SSE formation. Each treatment consisted of five replications and each experimental unit consisted of five explants.

### Collecting and Data Analysis

In the first experiment, observations were made on the percentage of PSE formation and the number of PSEs per responsive explant at 10, 13, 15, 18, and 21 weeks after culture (WAC), and at the second experiment, the observation was made on the number of SSEs per

PSE in each treatment. The effects of treatments were determined using variance analysis followed by DMRT at  $\alpha = 0.05$  using SPSS 20 Statistic software.

## RESULTS AND DISCUSSION

### Effect of PGRs Compositions on PSE Formation

The PGRs composition had a significant effect on the PSE formation at 10, 13, 15, 18, and 21 WAC. Medium containing kinetin  $0.125 \text{ mg l}^{-1}$  + TDZ  $25 \text{ } \mu\text{g l}^{-1}$  (M2) consistently showed the highest frequency of somatic embryogenesis (Figure 1A), whereas medium containing kinetin  $0.5 \text{ mg l}^{-1}$  (M1) showed the highest average number of PSEs per responsive explant (Figure 1B).

The kinetin singly (M1) or in combination with TDZ (M2, M3, M4), as a source of cytokinin in primary callus induction medium, showed a higher PSE response compared to TDZ singly (M5) which showed the lowest frequency of PSE (Figure 1A) and the lowest number of PSEs per responsive explant (Figure 1B). Previously, Ajjiah et al. (2014) reported the use of 2,4-D  $2 \text{ mg l}^{-1}$  + TDZ  $2.5 - 10.0 \text{ } \mu\text{g l}^{-1}$  could induce PSE of Sca 6 cacao clones but could not induce PSE of TSH 858 and ICS 13 clones. The results of this study indicate that kinetin and TDZ can synergize to induce somatic embryogenesis of MCC 01 and MCC 02 clones, although the use of kinetin singly, without TDZ, tends to provide better results for the frequency of PSE formation (Figure 1A).

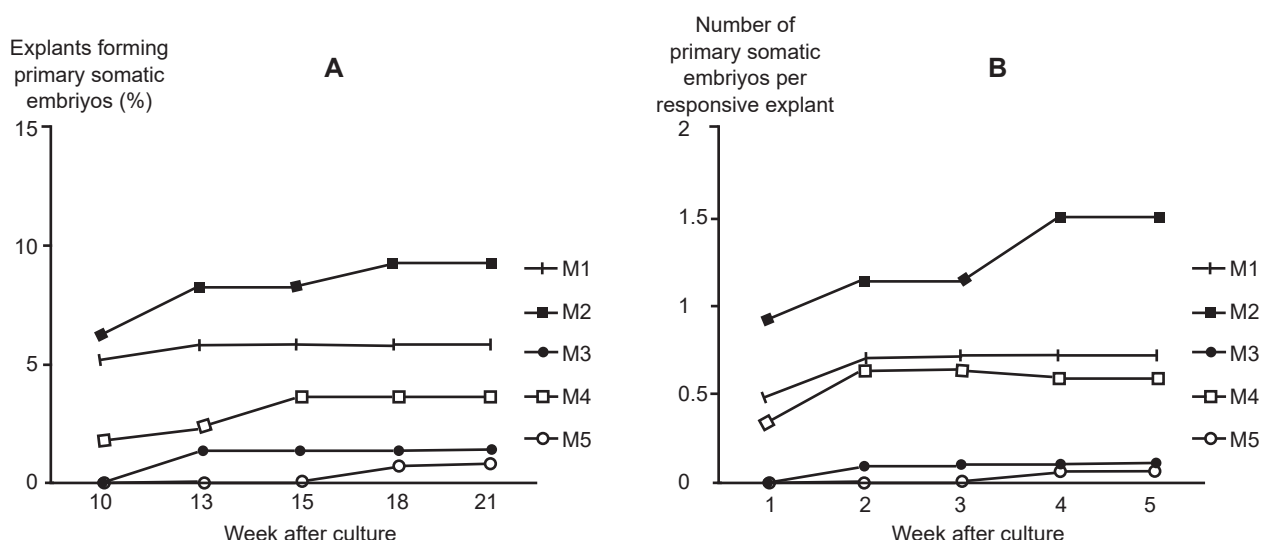


Figure 1. The primary somatic embryo formation (A) and the number of primary somatic embryos (B) of cacao on the different plant growth regulators; M1 = 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.5 \text{ mg l}^{-1}$ , M2 = 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.125 \text{ mg l}^{-1}$  + TDZ  $2.5 \text{ } \mu\text{g l}^{-1}$ , M3 = 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.125 \text{ mg l}^{-1}$  + TDZ  $5 \text{ } \mu\text{g l}^{-1}$ , M4 = 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.250 \text{ mg l}^{-1}$  + TDZ  $5 \text{ } \mu\text{g l}^{-1}$ , M5 = 2,4-D  $2 \text{ mg l}^{-1}$  + TDZ  $10 \text{ } \mu\text{g l}^{-1}$ .

The effectiveness of 2,4-D combined with kinetin to induce PSEs on some cacao genotypes has been reported. Kinetin was more effective for inducing cacao somatic embryogenesis of Cimanggu 1 accession compared to TDZ and BA (Ajijah and Hartati 2016) and BA (Kouassi et al. 2017) on some cacao genotypes. The stronger effect of kinetin compared to that of TDZ in inducing embryogenic callus is also reported in rubber (*Hevea brasiliensis*) (Kouassi et al. 2013) and coffee (*Coffea arabica* L.) (Gatica-Arias et al. 2008).

The effect of genotype and explant type as a single factor on the frequency of PSE formation and the number of PSEs per responsive explant were not statistically significantly different, while the interaction effects between genotype, explant type, and PGRs composition was found at the early (10 WAC) and end (18 WAC) periods of PSE formation, i.e., on the average percentage of PSE formation and the number of PSEs per responsive explant at 10 WAC and the average number of PSEs per responsive explant at 18 WAC (Table 2).

From the interaction effect, it can be seen that the PSE response is depending on the genotype, the explant type, and the PGR composition (Table 2). The highest frequency of PSE was obtained from staminoid explant of MCC 02 clone on the M2 medium, which is not statistically significantly different from that on the M1 medium (Table 2). The highest number of PSEs per

responsive explant was also obtained from staminoid explant of MCC 02 clone on the M1 medium (Table 2). Although staminoid explants showed a higher response of PSE compared to that of petals in MCC 02 clone, in MCC 01 clone, petal explants tend to show a higher response of PSE compared to that of staminoids (Table 2). M1 medium also tends to show a higher frequency of PSE and M4 medium tends to show a higher number of PSEs of MCC 01 clone, although not statistically different from other treatment (Table 2). There was no embryo formed from petal explant of MCC 01 clone on M3 medium and staminoid explant on M3 and M5 medium. There was also no embryo formed from petal explant of MCC 02 clone on M2 and M5 medium and staminoid explant on M3, M4, and M5 medium (Table 2). Likewise, the suitable explant for each clone is not the same, as well as the suitable medium for each explant and genotype. These results are in line with the previous studies which reported that the suitable explant for each cacao genotype is not the same (Ajijah et al. 2016; Garcia et al. 2016; Kouassi et al. 2017) and the suitable medium (PGRs level and composition) for each cacao genotype and each type of explant are also not the same (Issali et al. 2008; Quainoo and Dwomo 2012; Ajijah et al. 2014; Ajijah and Hartati 2016). Figure 2 and 3 showed the regeneration of MCC 01 and MCC 02 clones through PSE.

**Table 2. The effect of interaction between genotype, explant type, and plant growth regulators (PGRs) composition on the primary somatic embryos formation at 10 and 18 weeks after cultured (WAC).**

Genotype	Explant type	PGR composition	Explants forming primary somatic embryos (%)		Number of primary somatic embryos per responsive explant	
			10 WAC	18 WAC	10 WAC	18 WAC
MCC 01	Petals	M1	1.85 cd	3.52	0.17 c	0.67 b
		M2	10.44 b	12.67	0.53 bc	0.73 b
		M3	0.00 d	0.00	0.00 c	0.00 b
		M4	5.28 bcd	7.78	1.75 b	1.88 b
		M5	0.00 d	1.79	0.00 c	0.14 b
	Staminoids	M1	5.00 bcd	5.00	1.17 bc	1.17 b
		M2	0.00 d	4.86	0.00 c	0.40 b
		M3	0.00 d	0.00	0.00 c	0.00 b
		M4	0.00 d	3.13	0.00 c	0.75 b
		M5	0.00 d	0.00	0.00 c	0.00 b
MCC 02	Petals	M1	10.00 bc	10.00	1.00 bc	1.00 b
		M2	0.00 d	0.00	0.00 c	0.00 b
		M3	0.00 d	5.00	0.00 c	0.38 b
		M4	2.22 bcd	4.44	0.30 bc	0.50 b
		M5	0.00 d	0.00	0.00 c	0.00 b
	Staminoids	M1	20.00 a	20.00	4.00 a	9.00 a
		M2	22.20 a	22.20	1.50 bc	1.50 b
		M3	0.00 d	0.00	0.00 c	0.00 b
		M4	0.00 d	0.00	0.00 c	0.00 b
		M5	0.00 d	0.00	0.00 c	0.00 b

Means in the same column with different letters are significantly different according to DMRT ( $p < 0.05$ ).

M1 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.5 mg l<sup>-1</sup>, M2 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.125 mg l<sup>-1</sup> + TDZ 2.5 µg l<sup>-1</sup>, M3 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.125 mg l<sup>-1</sup> + TDZ 5 µg l<sup>-1</sup>, M4 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.250 mg l<sup>-1</sup> + TDZ 5 µg l<sup>-1</sup>, M5 = 2,4-D 2 mg l<sup>-1</sup> + TDZ 10 µg l<sup>-1</sup>.



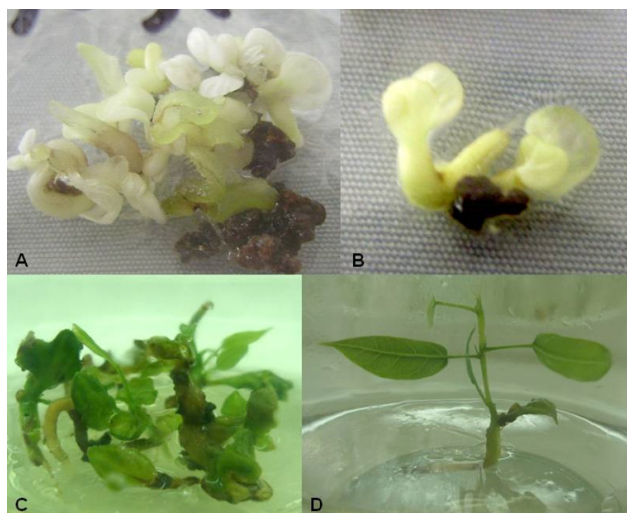


Figure 2. Regeneration of Indonesian cacao clone MCC 01 through primary somatic embryogenesis: (A) formation of primary somatic embryos, (B) cotyledonary stage of primary somatic embryos, (C) germination of somatic embryos, and (D) plantlet formation.

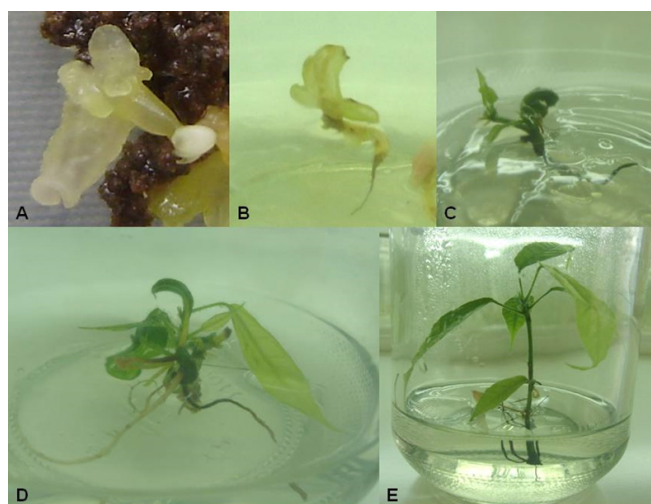


Figure 3. Regeneration of Indonesian cacao clone MCC 02 through primary somatic embryogenesis: (A) formation of primary somatic embryos, (B) cotyledonary stage of primary somatic embryos with taproot, (C) germination of somatic embryos, and (D, E) plantlet formation.

### Effect of Explant Types and PGRs Compositions on Secondary Somatic Embryo Formation

Even though the two clones showed a very low PSE response (Figure 1A), the MCC 01 clone showed a good SSE response (due to limited plant material, testing of SSE on MCC 02 clone was not carried out). The type of explant used in producing PSEs did not have a statistically significant effect on the SSE formation. However, PSEs induced from petal explant tend to produce a higher SSE formation (2.8 SSEs per PSE) compared to 1.33 SSEs per PSE from staminoid explant (Figure 4A). Petal explant also showed a higher PSE response compared to that of staminoid explant in the MCC 01 clone (Table 2). Consequently, petal explant is more recommended for producing both PSEs and SSEs of MCC 01 clone.

According to Jiménez (2005), the different responses of the explants are caused by the differences in endogenous hormone levels in each type of explant that affect the somatic embryogenesis response.

The composition of PGRs in the primary callus induction medium significantly affected the SSE formation. Even though M2 and M4 medium produced a higher number of PSEs than that of M1 in MCC 01 clone (Table 2), PSEs derived from callus induced using M1 medium produced a higher average number of SSEs (7.45 SSEs per PSE) compared to that from PSEs derived from callus induced using M2 and M4 medium (2.88 and 2.80 SSEs per PSE, respectively) (Figure 4B). The composition of PGRs in the primary callus induction medium was thought to affect the endogenous level of PGRs in PSEs, so that affected the

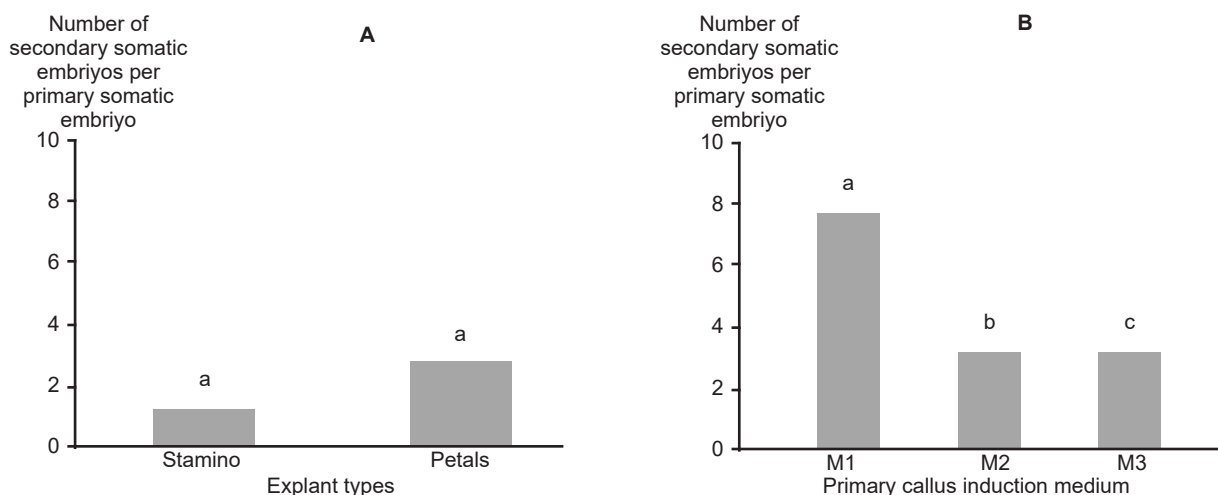


Figure 4. The formation of secondary somatic embryos of MCC 01 clone from primary somatic embryos induced from different explants (A) and on different plant growth regulators (B). M1= 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.5 mg l<sup>-1</sup>, M2 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.125 mg l<sup>-1</sup> + TDZ 2.5 µg l<sup>-1</sup>, M4 = 2,4-D 2 mg l<sup>-1</sup> + kinetin 0.250 mg l<sup>-1</sup> + TDZ 5 µg l<sup>-1</sup>.

SSE response. PGRs play an important role in determining the success of SSE induction (Silva et al. 2005; Karami and Deljou 2008; Pinto et al. 2008). Other factors are also reported affecting SSE response, i.e., basal salt medium (Pinto et al. 2008; Htay et al. 2013), light (Pinto et al. 2008), medium phases (Silva et al. 2005; Yang et al. 2013), and sucrose (Htay et al. 2013). As far as we know, this is the first report about the effect of explant type and PGRs used in inducing PSEs on the SSE response. From this study, it is known that PGRs used in inducing PSEs affected the response of SSE in cacao. Petal explant and M1 medium are more recommended for producing SSEs of MCC 01 clone.

SSE plays an important role in the cacao regeneration system for both mass propagation and genetic improvement. SSE in cacao commonly originated from a single cell, compared to PSE, which commonly originated from multicells (Maximova et al. 2002), so that SSE can effectively be used for *in vitro* selection, *in vitro* mutagenesis, or genetic transformation since it can produce solid regenerants without chimera. SSE also important for cacao mass propagation since it shows more fidelity compared to that in PSE (Rodríguez et al. 2010). SSE provides advantages compared to PSE, i.e., high multiplication rate, independence from explant sources, and repeatability (Yang et al. 2013).

In the case of MCC 01 and MCC 02 clones, developing PSE and SSE methods of the two cacao clones are important. MCC 01 and MCC 02 are two Indonesian superior cacao clones released by the Ministry of Agriculture, Republic of Indonesia in 2014. MCC 02 is the most preferred cacao clone cultivated by farmers at Sulawesi now since the clone has a high yield (3.13 ton ha<sup>-1</sup> year<sup>-1</sup>) and relatively resistant to vascular streak

dieback (VSD) and *Phytophthora*. Developing a somatic embryogenesis method for this clone is aiming for rapid and mass propagation. MCC 01 also showed a high yield characteristic (3.67 ton<sup>-1</sup> ha<sup>-1</sup> year<sup>-1</sup>), but relatively susceptible to pests and diseases. Therefore, genetic improvement for pest and disease resistances of this clone through conventional breeding or biotechnology approaches is required. Genetic improvement of cacao is hindered by its narrow genetic base and long life cycle, therefore availability of an efficient propagation method is essential to accelerate breeding programs (Wickramasuriya and Dunwell 2018). According to Yang et al. (2013), clonal propagation through SE could shorter time needed for breeding. Genetic transformation of the chitinase gene for enhancing resistance against a pathogen in cacao by using SSE and *Agrobacterium* has been reported by Maximova et al. (2006).

Some elite genotypes of cacao showed a low or an absence response to somatic embryogenesis induction (Daouda et al. 2019). Ajijah et al. (2016) also reported that the frequency of PSE in nine Indonesian cacao genotypes is varied between 0-67%. Results of this study showed that MCC 01 and MCC 02 have low responses of PSE (recalcitrant) which maximum frequency of PSE was only 12.67% with PSE number of 1.88 per responsive explant (MCC 01) and 22% with PSE number of 9 per responsive explant (MCC 02) (Table 2). However, developing method for SSE production of MCC 01 clone in this study could increase the multiplication rate of MCC 01 clone from PSE by 7 times. This method could be developed to other cacao clones with optimization in PGRs level and PGRs combination and selecting an appropriate explant.

## CONCLUSION

Genotypes, explant types, and plant growth regulators (PGRs) composition dependently affected primary somatic embryogenesis (PSE) response on cacao. The suitable explant and PGR composition for each cacao genotype are different. The best PSE response was obtained from staminoid explant of MCC 02 clone on medium containing 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.5 \text{ mg l}^{-1}$  (20%, 9 embryos). The PGRs composition used in inducing PSE affect the secondary somatic embryogenesis (SSE) response. The best medium for producing PSEs followed by SSE of MCC 01 clone is a medium containing 2,4-D  $2 \text{ mg l}^{-1}$  + kinetin  $0.5 \text{ mg l}^{-1}$ , with petal explant is more recommended compared to staminoids. This method can be used either for mass propagation or *in vitro* breeding and genetic transformation of MCC 01 or other cacao clones that are suitable for this method.

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