Optimation of Auxin and Cytokinin on Enhanced Quality and Weight of *Coffea liberica* Somatic Embryos

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Abstract

Coffea liberica is a variety of coffee that tolerant to marginal land, especially peatlands. One of propagation methods in *C. liberica* is somatic embryogenesis (SE) which producing large number of true-to-type plant seedlings in a short time. This research aimed at studying the effect of application of plant growth regulator (PGR) on quality and weight of somatic embryo of *C. liberica*. Somatic embryo in development stage was induced by Murashige and Skoog medium containing cytokinin as benzyl amino purin (BAP) and auxin as 2,4-dichlorophenoxyacetic acid (2,4-D). While cotyledonary embryo in germination stage was induced by Murashige and Skoog medium containing cytokinin (BAP) and auxins as 2,4-D, indole acetic acid (IAA) and naphthaleneacetic acid (NAA). The results showed that the application of auxins and cytokinins on development stage affected the formation of embryos, texture of calli, color of calli and embryos, and weight of somatic embryos. It also influenced the shoot and root formation, color and weight of germinating embryos of *C. liberica* at the germinating stage. During the development stage, addition of 1 mg.L^{-1} BAP in the absence of 2,4-D in MS medium produced the highest quality of somatic embryo of *C. liberica*. This medium also produced heaviest somatic embryos but with lighter callus. While in germination stage, all medium treatments produced a typical germinating embryo. *Coffea liberica* germinating embryo grew optimally on MS medium containing 0.5 mg.L^{-1} BAP as a single chemical or 0.5 mg.L^{-1} BAP in combination with 0.5 mg.L^{-1} IAA for shooting development. Whereas on rooting development, addition of 0.5 mg.L^{-1} NAA on MS medium produced an optimal germinating embryo. Moreover, germinating embryo of *C. liberica* recorded the highest in terms of dry weight on MS media with addition of 0.5 mg.L^{-1} BAP. Application of appropriate concentration of auxin and cytokinin is needed to support the formation of somatic embryos and germinating embryos.

Keywords: callus, somatic embryo, quality, weight, *Coffea liberica*

INTRODUCTION

Coffee is one of the commercial commodities in Indonesia that has great economic value in the global trading. In addition to *C. arabica* and *C. canephora,* there are two minor species that prominent in the trade of coffee beans and the cultivation, such as *C. liberica* and *C. dewevrei* (Sridevi & Giridhar, 2014). *Coffea liberica var. liberica* and *C. liberica var. dewevrei* are highly resistant to rust infections with good yield, require optimum temperature and light for cultivation (Lopes & Monaco, 1979). Its level of caffeine is about 1.2% (Ashihara & Crozier, 2001). *Coffea liberica* is cultivated for a special taste,

superior in agronomic character (simultaneous ripening period of fruit with larger size of seeds), low caffeine content, high adaptability to high salinity environment and peatland area (N'Diaye *et al.*, 2005). *Coffea liberica* is tolerant to grow on marginal land, especially peatlands which other varieties find it unsuitable. Indonesia and Malaysia are two countries with a large development area for *C. liberica* in addition to Philippines and Vietnam to smaller extent (Wintgens, 2004). Propagation on *C. liberica* uses several methods. True-to-type plant propagation is possible only through clonal propagation, which includes macropropagation methods such as grafting and cutting as well as micropropagation (Ahmed *et al.*, 2013). Micropropagation via somatic embryogenesis (SE) also permits the production of true-to-type plant on a large-scale within a short period of time (Etienne *et al.*, 2006).

Somatic embryogenesis is a developmental process where a plant somatic cell can differentiate to a totipotent embryonic cell that has the ability to give rise to embryo under appropriate conditions. This technology is important for woody plants that have a long life cycle and those that difficult to propagate by conventional methods (Isah, 2016). It can produce large numbers of true-to-type plant seedling in a short time (Etienne *et al.*, 2006). Since 2000s, SE has enabled and developed as propagation method for coffee clones. For *C. canephora* many research have been reported related to the application of this method (Muniswamy *et al.*, 2017). Similarly, the same reports are widely available on somatic embryogenesis of *C. arabica* (Ahmed *et al.*, 2013). Meanwhile, somatic embryogenesis in *C. liberica* still have a few reference (Ardiyani, 2015). Different to *C. arabica* and *C. canephora*, research on somatic embryogenesis of *C. liberica* has not been done much, especially on the application of plant growth regulators in culture medium.

One factor that affecting SE process is plant growth regulator (PGR). PGRs are specific to plant species (Santana-Buzzy *et al.*,

2007). They must be provided precisely and specifically in the context of type and quantity. Various type of PGRs produce different responses (Aga & Khillare, 2017). Types of PGRs those frequently used are auxins and cytokinins. The most familiar auxins are 2,4-dichlorophenoxyacetic acid (2,4-D), indole acetic acid (IAA) and naphthalenela-cetic acid (NAA), while cytokinins are kinetin, BA and benzyl amino purin (BAP). Auxin is required for proliferation of a proembryogenic mass (PEMs) but inhibits the development of PEMs into somatic embryos (Yang & Zhang, 2010). Cytokinin promotes plant growth, has antiaging potential, also protective effects for plants. Cytokinins can also enhance nutrition product, involved in cell growth and promoted differentiation. Auxins and cytokinins have a crucial role in regulation of organ regenerations, and the concentration ratio between these hormones are critical for determining specific organogenesis processes. In some plants, using auxins and cytokinis affect growth quality of somatic embryo. The study of *Medicago truncatula* also confirmed that higher auxin (NAA) level improved embryo quality (Nolan & Rose, 1998). There are various reports available for micropropagation of citrus varieties through callus formation and regeneration in the pre-sence of 2,4-D and BAP (Ali & Mirza, 2006). Culture medium containing growth regulators have a correlation with type of callus, somatic embryo multiplication frequency and the time spent exposed (Konan *et al.*, 2010).

Optimal quality performance and weight of somatic embryo influence their potentiality to develop to plantlet. Guan *et al.* (2016) stated that maturation and quality of somatic embryos are further limiting factors in converting embryos into plants. One of limiting factors that influenced the maturation and quality of somatic embryo is PGR. Therefore, this research discuss about the effect of application of several concentration of auxins and cytokinins to enhance the quality and weight of somatic embryo *C. liberica*.

MATERIALS AND METHODS

Preparation explant and media

This research was done at Tissue Culture Laboratory, Indonesian Coffee and Cocoa Research Institute, from May to December 2019. This research used embryogenic calli of *C. liberica* for development stage, and cotiledonary embryos for germinating stage. These explants were cultured on Murashige-Skoog (MS) medium containing auxin and cytokinin. For cytokinin benzyl amino purin/ BAP was used, while for auxins 2,4,-dichlorophenoxy acetic acid/2,4-D, indole acetic acid/IAA and naphthaleneacetic acid/NAA were used. This medium is also combined with vitamin B5, gelling agent/gelrite and sucrose. For observation, the inverted Olympus SZ51 stereo microscope was used. Tissue culture process is carried out in the laminar air flow (LAF) Esco-ULFA-AHC-601.

Development Stage

During the development stage, 1 mg of embryogenic calli of *C. liberica* were cultured in medium treatments. Culture preparation on this research used Murashige-Skoog medium, 30 $g.L^{-1}$ sucrose, vitamin B5 and addition of 1 $mg.L^{-1}$ BAP combined with 0.5, 1, 1.5, 2 $mg.L^{-1}$ 2,4-D and without addition of 2,4-D (Berthouly & Michaux-ferriere (1996). Embryogenic calli were cultured in petridish (diameter 50 mm). Each treatments applied 30 replications. All treatments medium were solidified with gelrite 3 $g.L^{-1}$, and pH was adjusted to 5.6–5.8. This medium were sterilized by autoclave at 120°C for 20 min. These cultures were placed in the dark incubation rooms at 25–28°C and 80% humidity. This cultures were incubated for 12 weeks and sub-cultured in the same medium every 2 weeks. In this stage, observation were done for embryos formed, texture and color of calli as well as embryos. Embryo formed and color of embryo were used to determine embryos quality, so that can be used as an explant during the germination stage. Weight of callus, cotyledonary embryos and total embryos also observed by analytical scales Mettler Toledo Xs205du.

Germination Stage

In germination stage, the 2 mm length of cotiledonary embryos were used as explants. Culture medium in germination stage consisted of Murashige-Skoog medium as basic media, vitamin B5, 30 $g.L^{-1}$ sucrose, 4 $g.L^{-1}$ gelling agent (gelrite), and addition of PGR, such as 0.5 $mg.L^{-1}$ BAP (1), 0.5 $mg.L^{-1}$ IAA (2), 0.5 $mg.L^{-1}$ NAA (3), 0.5 $mg.L^{-1}$ BAP + 0.5 $mg.L^{-1}$ NAA (4) and 0.5 $mg.L^{-1}$ BAP + 0.5 $mg.L^{-1}$ IAA (5). The cotyledonary embryos were cultured in petridish (160 mm in diameter), and each of treatments were replicated for 10 times. The cotyledonary embryos were placed in the growth room at constant temperature of 25–28°C, 80% humidity and 8-h dark/16-h light photoperiod for 12 weeks. Quality parameters on the germination stage observed were the formation of shoot and root, and color of germinating embryos. Whilst, size and weight of germinating embryo were also observed in this research. For the dry weight, data were obtained from 5 destructive samples of germinating embryos for each treatments. The dry weight of germinating embryos were obtained from the samples dried at 80°C.

Data Collection and Analysis

This research observed somatic embryo quality and weight during the development and germination stages. In the development stage, there were 5 treatments of PGR, namely, 1 $mg.L^{-1}$ BAP combined with 0.5, 1, 1.5, 2 $mg.L^{-1}$ 2,4-D and without addition of 2,4-D. Whereas in germination stage, there were also 5 treatments, such as 0.5 $mg.L^{-1}$

BAP, 0.5 mg.L^{-1} IAA, 0.5 mg.L^{-1} NAA, 0.5 mg.L^{-1} BAP + 0.5 mg.L^{-1} NAA and 0.5 mg.L^{-1} BAP + 0.5 mg.L^{-1} IAA. This assessment on the somatic embryo quality and weight were done qualitatively by determining the formation of callus and embryos, callus and embryo color and texture of callus. Quantitative assessment were done by weighing of callus, cotyledonary embryos and total embryos. Embryos quality was divided into two categories, namely, good quality embryos, that is white cotyledonary embryo, and the yellowish unvigorous embryos. In germination stage, qualitative variable were done through the formation shoot and root and color of germinating embryos. Whilst quantitative assessment were using the size and weight of germinating embryos. This research employed a completely randomized design (CRD) with 5 treatments of combination of auxins and cytokinins in several concentrations during the development stages. Each treatments were replicated for 30 times. For germination stages, it also employed a CRD with 5 treatments and 10 replications. Data were analyzed statistically using SPSS (version 17.0). Analysis of variance was used to test if there any significant difference among the treatments, and followed by Duncan test (0.05). The P value of <0.05 was considered as significant.

RESULTS AND DISCUSSION

Embryogenic callus of *C. liberica* responded positively to all medium of cultures. It grew and developed into somatic embryos (Figure 1). In this stage, cells on embryogenic callus developed individually become somatic embryos. In somatic embryo-genesis, the addition of PGR stimulated callus to develop and affect the quality of somatic embryos.

Callus on Development Stage

Embryogenic callus of *C. liberica* in MS medium containing BAP without addition of 2,4-D demonstrated a different quality from embryogenic callus cultured in the medium containing of BAP and 2,4-D (Table 1).
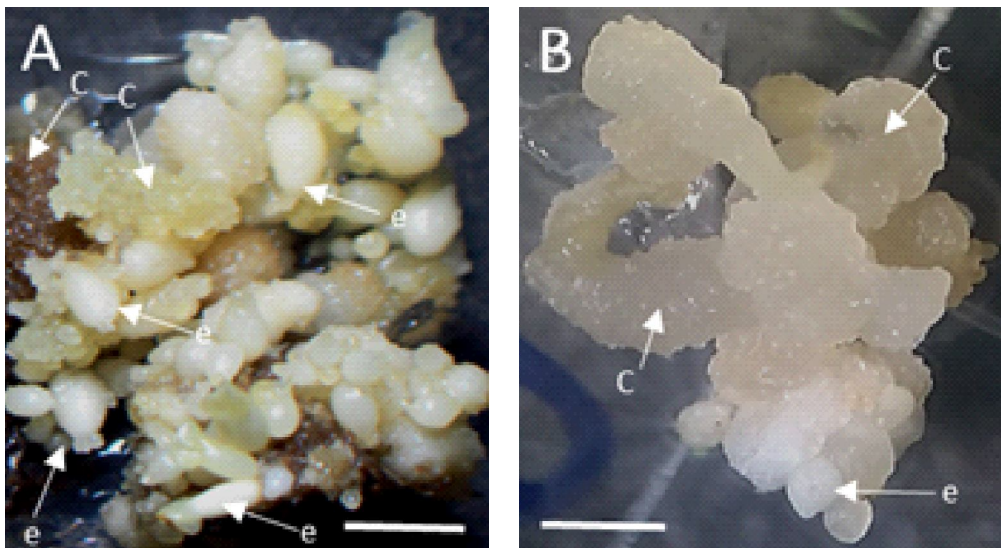

Figure 1.   Somatic embryo *C. liberica* in (A) MS medium with adding 1 mg.L^{-1} BAP without 2,4-D, (B) MS medium with adding 1 mg.L^{-1} BAP + 2 mg.L^{-1} 2,4-D. e = embryo; c = callus, Bar : 1 mm

The results showed that after 12 weeks of culture (W12), callus of *C. liberica* was formed in all treatments medium. The number of callus on MS medium containing 1 mg.L^{-1} BAP individually and callus on MS medium containing 1 mg.L^{-1} BAP and 0,5 mg.L^{-1} 2,4-D reduced in 8th weeks after culture, which is not observed for other treatments. MS medium containing 1 mg.L^{-1} BAP and 1–1.5 mg.L^{-1} 2,4-D produced brown and friable calli. It means the calli still embryogenically and potentially develop into somatic embryo. While on MS medium supplemented with 1 mg.L^{-1} BAP and 2 mg.L^{-1} 2,4-D produced brown and compact calli. Likewise, MS medium containing 1 mg.L^{-1} BAP without addition of 2.4 D, and MS medium containing 1 mg.L^{-1} BAP and 0.5 mg.L^{-1} 2,4-D produced aqueous calli. The formation of compact and aqueous calli showed a decrease in the quality and performance of embryogenic cells. Embryogenic calli were friable, light, glossy, and consisted of separated individuals, while non embryogenic calli were compacted, less friable, and hard clustered structure with dark brown color (Ardiyani, 2015). A 2,4-D is one of the most commonly auxins used in plant tissue culture practices. Auxins are well known for the induction of embryogenesis either as a single chemical or in combination with different hormones. This findings are in accordance with result of Kazmi *et al.* (2015), who reported the culture medium with 2.5 mg.L^{-1} 2,4-D and 0.5 mg.L^{-1} BAP in *Citrus reticulata blanco* L. produced friable and embryogenic callus. In *Moringa oleifera*, MS medium supplemented with 11.31 µM 2,4-D and 2.21 µM BAP transformed embryo explants directly to friable callus on periodically sub-cultured (Devendra *et al.*, 2012). Vondrákov *et al.* (2011) also confirmed that cytokinins and auxins were suitable for induction of the embryogenic suspensor mass (ESM) from the mother explants. Kaviani (2013) reported that in *Camellia sinensis* L., the most effective concentration for somatic embryo initiation were obtained from the addition of 1 µM of 2,4-D. Generally, auxins promote cell division and root differentiation on tissue culture. Moreover, auxins are also used for inducing cell division, cell elongation, and formation of callus.

Somatic Embryo on Development Stage

In the development stage, MS medium supplemented with BAP and 2,4-D are also produced somatic embryos. Somatic embryos of *C. liberica* were able to produce from all treatment mediums. Medium of MS containing 1 mg.L^{-1} BAP in the absence of 2,4-D produced more somatic embryos than different treatments (Table 2).

Table 1.   Quality of embryogenic calli *C. liberica* on MS medium with BAP and 2,4-D at 0, 4, 6, 8, 10 and 12 weeks after culture

Treatments	Callus formed					Color of callus					Texture of callus W12
	W4	W6	W8	W10	W12	W4	W6	W8	W10	W12	
1 mg.L^{-1} BAP (without 2,4-D)	++	+	+	+	+	B	B	PY	PY	PY	aqueous
1 mg.L^{-1} BAP + 0.5 mg.L^{-1} 2,4-D	++	++	+	+	+	B	B	PY	PY	PY	aqueous
1 mg.L^{-1} BAP + 1 mg.L^{-1} 2,4-D	++	++	++	++	++	B	B	B	B	B	friable
1 mg.L^{-1} BAP + 1.5 mg.L^{-1} 2,4-D	++	++	++	++	++	B	B	B	B	B	friable
1 mg.L^{-1} BAP + 2 mg.L^{-1} 2,4-D	++	++	++	++	++	B	B	B	B	B	compact

Notes: + = few calli, ++ = profuse calli, B = brownish color, PY = pale yellow color.

Table 2 showed that embryogenic callus on MS medium containing 1 mg.L⁻¹ BAP in the absence of 2,4-D produced a lot of somatic embryos than MS medium containing BAP and 2,4-D. The quality of somatic embryos were described at the respective developmental stages. During embryogenesis, clear developmental stages were recognized. In 12 weeks after culture somatic embryo in globular, heart, torpedo and cotyledonary stages were formed from all treatment mediums. Whilst, white somatic embryos indicated an initiation of germination (Figure 2A). Whereas yellow somatic embryos showed a bit weaker (Figure 2B). These somatic embryos produced from MS medium containing 1 mg.L⁻¹ BAP and 1.5–2 mg.L⁻¹ 2,4-D.

Addition of PGRs in the culture medium affected the formation of somatic embryos *C. liberica*. This result indicates a reduced or an inhibited development stage during the formation of somatic embryos *C. liberica*. Vondrákov *et al.* (2011) indicated that development of embryogenic suspensor mass into mature embryos can be induced by transfer it onto medium lacking of auxins and cytokinins. In different samples, such as cucumber and carrot, removal of 2,4-D and decreasing in IAA limit of callus proliferation leading to establishment of pre-globular embryos and development of somatic embryo on the same medium (Ogata *et al.*, 2005).

Addition of BAP in the absence of 2,4-D produced optimal somatic embryos of *C. liberica* formation. In different cases, such as on *C. arabica*, the highest percentage of somatic embryos development was obtained from MS medium containing 4.0 mg.L⁻¹ BAP (Ahmed *et al.*, 2013). Nakamura *et al.* (1992) reported the highest somatic embryos of *C. arabica* obtained from MS medium supplemented with 1.0 mg.L⁻¹ BAP as the sole PGR. Cytokinins induce cell division and differentiation.

Callus and somatic embryo weight

Addition of BAP and 2,4-D on *C. liberica* development medium also affected callus and somatic embryo weight (Table 3). The results

Table 2. Quality of somatic embryo *C. liberica* on MS medium with BAP and 2,4-D at 0, 4, 8, 10 and 12 weeks (W) after culture

Treatment	Embryo					Embryo step					Embryo color				
	W4	W6	W8	W10	W12	W4	W6	W8	W10	W12	W4	W6	W8	W10	W12
1 mg.L⁻¹ BAP + 0 mg.L⁻¹ 2,4-D	-	+	+	++	+++	E	G	G,H	G,H,T,C	G,H,T,C	-	W	W	W	W
1 mg.L⁻¹ BAP + 0.5 mg.L⁻¹ 2,4-D	-	+	+	++	+++	E	G	G	G,C	G,H,T,C	-	W	W	W	W
1 mg.L⁻¹ BAP + 1 mg.L⁻¹ 2,4-D	-	+	+	++	+++	E	G	G	G,H	G,H,T,C	-	W	W	W	
1 mg.L⁻¹ BAP + 1,5 mg.L⁻¹ 2,4-D	-	+	+	+	++	E	G	G	G,H	G,H,T,C	-	Y	Y	W,Y	W
1 mg.L⁻¹ BAP + 2 mg.L⁻¹ 2,4-D	-	+	+	+	++	E	G	G	G	G,H,T,C	-	Y	Y	W	W

Note: - = no embryo, + = 1-5 embryos, ++ = 6-10 embryos, +++ = >10 embryos, E = embryogenic calli, G = globular stage, H = heart stage, T = torpedo stage, C = colyledon stage, W = white color, Y = yellowish color.
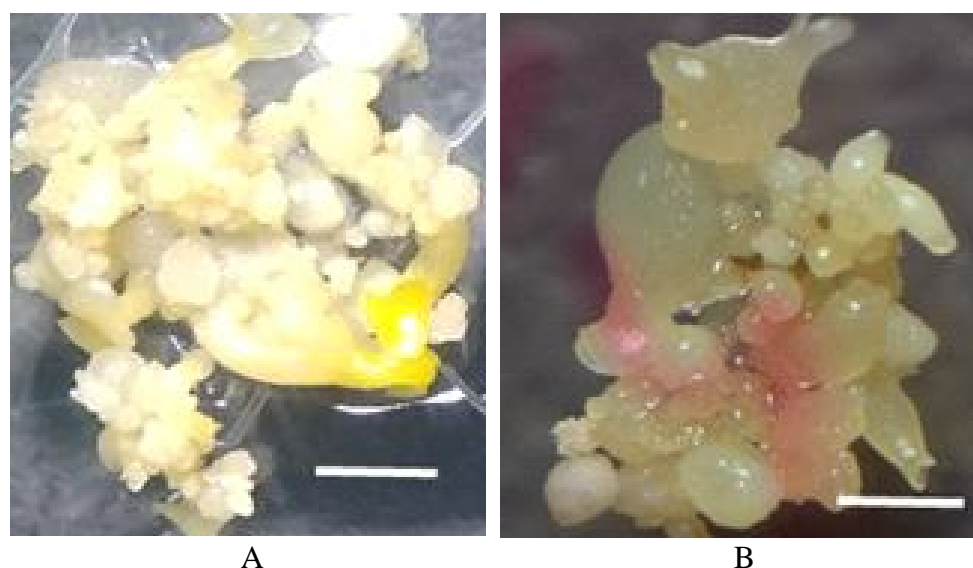
Figure 2.   Somatic embryo on MS medium with adding BAP without 2,4-D (A), somatic embryo on MS medium with adding BAP and 2,4-D (B) at 12 weeks after culture; Bar : 1 mm

were significantly different among treatment at 12 weeks after culture.

Embryogenic callus on MS medium containing 1 mg.L^{-1} BAP without 2,4-D produced heaviest somatic embryos but with lighter calli. These results were significantly different from other treatments. The addition of 1 mg.L^{-1} BAP and 0.5-2 mg.L^{-1} 2,4-D reduced somatic embryos weight, but increased for calli. Nutrient absorption on somatic embryos is better than the same absorption for calli. Additionally, the application of 1 mg.L^{-1} BAP in MS medium individually stimulated somatic embryos formation, but conversely inhibit callus formation. Addition of 1 mg.L^{-1} BAP and 2,4-D also affected the cotyledonary embryos weight. Heavier cotyledonary embryos were resulting in the better growth performance than the lighter embryos. The data in the Table 3 showed that weight of cotyledonary embrio on MS medium containing 1 mg.L^{-1} of BAP in the absence of 2,4-D obtained significantly different results in compare to other treatments (51.75 mg). However, the development of somatic embryo is more important than callus. Somatic embryos with optimal quality are required during the germination stage. Similar trend was

observed, callus induction on auxin containing medium and embryo development on auxin free medium is being used as standard method in a wide range of plant species (Raghavan, 2004).

Germination Stage

Somatic embryos have been developed on germination medium containing fully Murashige-Skoog medium with addition of auxins and cytokinins. This PGRs were applied as a single chemical or in combination with different hormones. The result of this research showed a different quality of germinating embryo of *C. liberica* in each of treatment (Table 4).

The study reported that all medium treatments produced normal germinating embryos. Roots and shoots have developed and grown up to planlet. *Coffea liberica* roots emerged at 12 weeks after culture in all medium treatments, whereas the shoot appeared at 4 weeks after culture in MS medium containing 0.5 mg.L^{-1} BAP. This medium supported for shoot development and produced higher number of shoots than other treatments.

The results also showed that at 12th weeks after culture, the color of germinating embryo changed from white to greenish. Greenish embryos expressed a high quality and potential embryos to continuously grow up to a plantlet. The color changing on germination embryos had been caused by 8-h dark/16-h light photoperiod. Cybularz-Urban *et al.* (2007) stated that in Orchidacae, light intensity has strong energy to affect morphology including the color of germinating embryos. Light energy is absorbed by chlorophyll, a photosynthetic pigment of the plant, and used for photosynthesis processes. Photosynthesis is the process in which light energy is converted into chemical energy. Using the energy of light, carbohydrates such as sugars are synthesized from carbon dioxide and water. This energy is needed for growth of embryo. Therefore, photoperiod affects the development of germinating embryo optimally.

In addition, size of root and shoot *C. liberica* were also influenced by addition of auxins and cytokinins. *Coffea liberica* germinating embryos grew optimally on MS medium supplemented with 0.5 mg.L^{-1} BAP and 0.5 mg.L^{-1} BAP combining with 0.5 mg.L^{-1} IAA for shooting development. Whereas on rooting development, adding 0.5 mg.L^{-1} NAA in MS medium produced optimal germinating embryo (Table 5).

Addition of auxins and cytokinins on germination medium provided different length of shoot. Culture medium with 0.5 mg.L^{-1} BAP and 0.5 mg.L^{-1} BAP combining with 0.5 mg.L^{-1} IAA resulted in the longest shoot among the treatments. Supplemented BAP individually or combining with another PGRs increased the length of shoot. It occurred because of division, development and elongation processes of apical meristem. Similar trend was observed in *Aquilaria malaccensis* culture, 0.5 mg/L BAP increasing the shoot length (Wardatutthoyyibah *et al.*, 2015). The interaction of auxin and cytokinin affect several processes in plants, such as the initiation and maintenance of plant tissue (Hua Su *et al.,* 2011). According to Fereol *et al.* (2002), auxins generally inhibit shoot development, whereas the combination of cytokinins and auxins supporting shoot and leaf development. Combination of auxins and cytokinins are also affecting on germinating embryo elongation. The optimal concentrations combination of auxins and cytokinins, stimulate protein synthesis in plant tissue then stimulating cell division and elongation, which affect length of plantlet (Widiastoety, 2014).

Table 3. The effect of BAP and 2,4 D on weight of embryo and callus *C. liberica* from 1 mg callus at 12nd weeks of age

Medium culture	Callus weight (mg)	Total weight embryo (mg)	Weight of embryo cotyledonary (mg)
1 mg.L^{-1} BAP (without 2,4-D)	5.50±0.45 [ab]	64.491±2.43 c	51.76±2.33 [d]
1 mg.L^{-1} BAP + 0.5 mg.L^{-1} 2,4-D	5.34±0.79 [a]	36.224±1.71 b	28.07±1.55 [c]
1 mg.L^{-1} BAP + 1 mg.L^{-1} 2,4-D	7.23±0.77 [c]	17.456±1.01 a	11.53±0.99 [a]
1 mg.L^{-1} BAP + 1.5 mg.L^{-1} 2,4-D	6.04±0.70 [b]	17.715±1.04 a	12.11±0.94 [a]
1 mg.L^{-1} BAP + 2 mg.L^{-1} 2,4-D	9.14±0.79 [d]	18.188±0.53 a	13.63±0.51 [b]

Note: Means followed by the same letters are not significantly different from each other at (p<0.05) determined by Duncan's multiple range test (DMRT).

Table 4. Morphology of germinating embryo *C. liberica* at 0, 4, 8, and 12 weeks (W) after culture

Treatments	Root in				Shoot in				Embryos color in			
	W0	W4	W8	W12	W0	W4	W8	W12	W0	W4	W8	W12
0.5 mg/L^{-1} BAP	-	-	-	+	-	+	+	++	W	W	Y	G
0.5 mg/L^{-1} IAA	-	-	-	+	-	-	+	+	W	W	Y	YG
0.5 mg/L^{-1} NAA	-	-	-	+	-	-	+	+	W	W	Y	G
0.5 mg/L^{-1} BAP + 0.5 mg/L^{-1} NAA	-	-	-	+	-	-	-	+	W	Y	Y	G
0.5 mg/L^{-1} BAP + 0.5 mg/L^{-1} IAA	-	-	-	+	-	-	+	+	W	W	Y	G

Notes: Root (- = root nodular, + = root formed); shoot (- = shoot nodular, + = 1 pair leaves, ++ = > 1 pair leaves); W = white, Y = yellowish, G = greenish, YG = yellowish green.

Likewise, addition of PGRs also effected in rooting development. Addition of 0.5 mg.L^{-1} NAA resulted in the most extensive root than the others. While addition of 0.5 mg.L^{-1} BAP resulted in the shortest root's length than other treatments. Auxin and cytokinin have an opposite function in the root meristem. Auxin promoted development of root meristems. Adding exogenous auxin increased root size, while adding cytokinins inhibited root growth (Chapman & Estelle, 2009). Hasanah & Setiari (2007) mentioned that the use of auxin in *Pogostemon cablin* had an optimal effect on the number of roots and root's length. This indicated that auxin triggers root initiation and promotes root elongation.

Germinating Embryo Weight

Besides affected on size of the embryo, supplemented of PGRs also affected on *C. liberica* germination embryo weight. Addition 0.5 mg.L^{-1} BAP produced the highest dry weight of germinating embryo. Whereas MS medium with addition of auxin individually resulted in the lowest of dry weight of germinating embryo (Table 6).

The data showed that after 12 weeks of culture, germination of embryo of *C. liberica* had highest dry weight on MS medium containing 0.5 mg.L^{-1} BAP (1.86 mg). Increasing of dry weight in the embryo somatic with the addition of BAP also occurs in different plants. Zubo *et al.* (2008) mentioned that cytokinins increased the number of carbon sequestration to the function of photosynthesis. In addition, cytokinins on media culture also stimulated transcription process in barley plants. In other plants, application of 5 mg.L^{-1} BAP was sufficient to increase dry weight of *Epipremnum aureum* L. compared with control. This study also pointed out that addition of BAP increase relative growth rate (RGR) (Benedetto *et al.*, 2015). Application of BAP in the medium resulted in significantly different of dry weight compared with application of auxins and cytokinins separately or in combination.

Table 5. The effect of auxin and cytokinin in MS medium on the size of germinating embryo of *C. liberica* at 12th weeks after culture

Medium culture	Shoot length (mm)	Root length (mm)
0.5 mg.L^{-1} BAP	5.20 ± 0.17[d]	0.81 ± 0.18[a]
0.5 mg.L^{-1} IAA	4.00 ± 0.35[c]	1.11 ± 0.23[b]
0.5 mg.L^{-1} NAA	2.98 ± 0.20[a]	3.13 ± 0.29[e]
0.5 mg.L^{-1} BAP + 0.5 mg.L^{-1} NAA	3.22 ± 0.09[b]	2.12 ± 0.19[d]
0.5 mg.L^{-1} BAP + 0.5 mg.L^{-1} IAA	5.02 ± 0.20[d]	1.32 ± 0.16[c]

Note: Means followed by the same letters are not significantly different from each other at P<0.05 determined by Duncan's multiple range test (DMRT).

Table 6. The effect of adding auxin and cytokinin in MS medium on the weight of germinating embryo of *C. liberica* at 12th weeks after culture

Medium culture	Fresh weight (mg)	Dry weight (mg)
0.5 mg.L^{-1} BAP	14.94 ± 0.45[d]	1.86 ± 0.055[d]
0.5 mg.L^{-1} IAA	5.05 ± 0.36[a]	0.631 ± 0.044[a]
0.5 mg.L^{-1} NAA	5.09 ± 0.42[a]	0.636 ± 0.052[a]
0.5 mg.L^{-1} BAP + 0.5 mg/L^{-1} NAA	7.06 ± 0.48[b]	0.882 ± 0.059[b]
0.5 mg.L^{-1} BAP + 0.5 mg/L^{-1} IAA	12.30 ± 0.47[c]	1.537 ± 0.058[c]

Note: Means followed by the same letters are not significantly different from each other at (p<0.05) determined by Duncan's multiple range test.

CONCLUSIONS

Application of auxin and cytokinin on development stage affected quality and weight of somatic embryos. Auxin and cytokinin require a different concentration to obtain an optimal somatic embryo and germination embryo. In the development stage, precise compositions of auxin and cytokinin are needed to support somatic embryos. Somatic embryos on MS medium containing 1 mg.L^{-1} BAP without 2,4-D produced somatic embryo *C. liberica* with an optimal quality as well as heaviest somatic embryos. Whilst, auxins and cytokinins also influenced the quality and weight of geminating embryos of *C. liberica* on gemination stage. *C. liberica* germinating embryo growth optimally on MS medium supplemented with BAP individually or MS medium with 0.5 mg.L^{-1} BAP combining with 0.5 mg.L^{-1} IAA for shooting development. Whereas on rooting development, addition of 0.5 mg.L^{-1} NAA in MS medium culture produced optimal germinating embryos. Moreover, germinating embryo of *C. liberica* on MS medium containing 0.5 mg.L^{-1} BAP had a highest dry weight.

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