Strategy Propagation of *Coffea arabica* L. by Tissue Culture Techniques

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Abstract. The research of in vitro callus induction on arabica coffee (Coffea arabica L.) with 2.4 D, BAP, and NAA, was conducted in Biotechnology and Genetics Laboratory, Tissue Culture Unit, Department of Biology, Faculty of Mathematics and Natural Sciences. Tadulako University. The purpose of this research was determine a callus induction method for Coffea arabica L. in vitro propagation using a variety of mediums. A completely randomized Design (CRD) with 6 treatments and 3 replications was used in this experimental. BAP 1 ppm + NAA 0.2 ppm, BAP 1.5 ppm + NAA 0.2 ppm, BAP 2 ppm + NAA 0.2 ppm, 2.4 D 1 ppm + BAP 0.2 ppm, 2, 4 D 1.5 ppm + BAP 0.2 ppm, and 2.4 D 2 ppm + BAP were the different treatments. 0.2 ppm in. Based on the results, it can be determined that 2.4D 2 ppm + 0. 2 ppm BAP with a 100% callus percentage was the proper and effective of treatment. With a crumb texture and a white callus appearance.

Keywords 2.4 D, BAP, NAA, Callus, Coffea

I. INTRODUCTION

Coffee is one of the plantation commodities that has a fairly high economic value compared to other plantation crops, coffee also acts as a source of foreign exchange for the country. The success of coffee agribusiness requires the support of all relevant parties so that the competitiveness of coffee in Indonesia can compete in the world market (Rahardjo, 2012).

Central Sulawesi is one of the coffee producing regions. The plantation area is 8.90 ha in 2018, 9.70 ha in 2019 and 9.90 ha in 2020 (BPS, 2020). This commodity has a competitive market export value, both domestic and foreign markets. In addition, coffee serves as a mixture of food and cosmetics. This has been widely applied and used by industries that manage coffee as a supporting material for types of food and other supporting materials (Yahya, 2016).

Conventionally, coffee plants can be propagated vegetatively (grafting and grafting) and generative (seeds). However, in both methods of breeding, there are still some weaknesses (Tahardi et. al. 1997). Improvements in Arabica coffee quality and seedling techniques that are possible more quickly and efficiently are through in vitro culture techniques (Priyono, 1993). In vitro culture is a technique for growing plant cells or tissues into whole. The advantages of in vitro culture techniques, among others, are as an effective and efficient method for mass reproduction of plants that have high economic value, can be incubated/stored in a culture environment for a long period of time, and can be used as material to support genetic transformation in order to obtain varieties. new (Gunawan, 1987).

In this research, I will use Coffee arabica as an explant in vitro culture due to the production of arabica coffee in the world of marketing is a coffee that has a very high number of fans this is because Arabica coffee has the advantage of a distinctive taste and aroma compared to other coffees. In addition, in Sulawesi, in particular, Central Sulawesi coffee production is still minimal due to the process of long production time and produce less production so that farmers' attention is more focused on robusta coffee. This is why it should be conducted in vitro culture research on arabica coffee in Central Sulawesi.

II. METHOD

Plant materials used as explants were young leaves of *Coffea arabica* L., Murashige and Skoog (MS) media, 2,4-D, NAA, BAP, agar, sucrose, aquades, HCL, KOH, 70% alcohol, spritus, fungicides. , and detergents.

The method used is Completely Randomized Design (CRD),with 6 treatments and 3 replications so that the number of treatments observed was 18 bottles.

No	Treatment	Media Composition
1.	C1	MS +BAP 1 ppm+ NAA 0.2 ppm
2.	C2	MS +BAP 1.5 ppm+ NAA 0.2 ppm
3.	C3	MS + BAP 2 ppm+ NAA 0.2 ppm
4.	C4	MS + 2.4 D 1 ppm + BAP 0.2 ppm

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5.	C5	MS + 2.4 D 1.5 ppm + BAP 0.2 ppm
6.	C6	MS + 2.4 D 2 ppm + BAP 0.2 ppm

Table 1. Tested planting medium

The working steps in the sterilization of Arabica coffee explants were the young leaves were taken, and the young stems were washed in running water using detergent for 3 repetitions to remove dirt on the leaf surface. Then weigh 2 g of the functionala solution and dissolve it with sterile distilled water and then soak the cleaned young leaves into the fungicide for 10-15 minutes to remove the fungus that is inside and on the leaf surface. Next, prepare 70% alcohol and sterile distilled water. After the explants had been soaked, they were then dipped in 70% alcohol for 3-5 seconds and then rinsed again using sterile water 3 times.

Culture bottles that have been planted with seedlings in a dark room until the callus is successfully induced with temperature 24-26^{0}C. The research parameters:

a. Days of callus after planting (DAP)

Observations of explants that had induced callus were carried out every day, starting from the day after planting.

b. Callus color

Visual observation of callus color was carried out in the second week after callus grew which was observed by at least 3 people because the visual appearance of each person's color was sometimes different.

c. Callus Texture

Callus texture was observed at the end of the study.

III. Results and Discusion

a. Days of callus after planting (DAP)

Callus growth responses at concentrations of C1, C2, C3, C4, C5, and C6 had different responses because not all explants could induce callus. In treatments C1, C3, C5, C6 could induce callus formation, while in treatments C2 and C4 only swelling of the explants and could not induce callus formation. The fastest treatment in inducing callus can be seen in the image below.

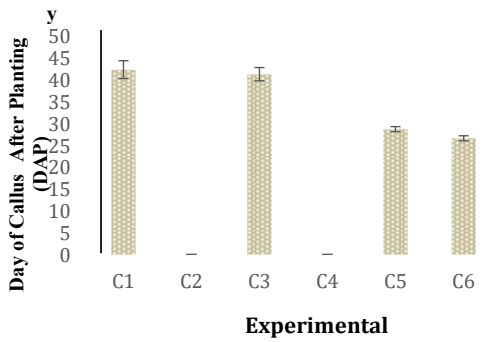

Fig.1 Callus growth rate in each treatment.

b. Callus color

Callus color was seen based on the physical form of the callus produced in each treatment by looking at the color of the callus on the last day of observation. Callus color on *Coffea arabica* L. explants can be seen in the table below.

Table. 1 Callus Color of *Coffea arabica* L. In various treatments

Treatment Concentration	Test		
	1	2	3
C1 (BAP 1 + NAA 0.2) ppm	Yellowish white	Brownish yellow	Yellowish white
C2 (BAP 1.5 + NAA 0.2) ppm	-	-	-
C3 (BAP 2 + NAA 0.2) ppm	Yellowish white	Chocolate	Chocolate
C4 (2.4D 1 + BAP 0.2) ppm	-	-	-
C5 (2.4D 1.5 + BAP 0.2) ppm	White	Chocolate	Yellow
C6 (2.4D 2 + BAP 0.2) ppm	White	Yellow	Brownish yellow

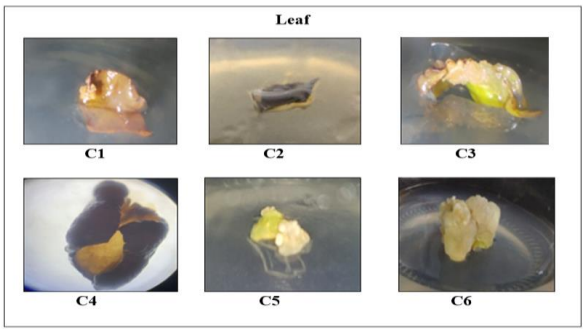

Fig. 2 Callus color of *Coffea arabica* L. treatment of C1, C3, C5, C6. Treatment C2 and C4 did not grow.

Callus Texture

Callus texture was observed based on the physical form produced in each treatment by looking at the last day of observation. can be seen in table 2 The average callus texture produced by each treatment tested was of the crumb type, this showed a good response.

Tabel. 2 Callus Texture of *C. arabica* L. In various treatments

Treatment Concentration	Test		
	1	2	3
C1 (BAP 1 + NAA 0.2) ppm	Crumb	Compact	Crumb
C2 (BAP 1.5 + NAA 0.2) ppm	-	-	-
C3 (BAP 2 + NAA 0.2) ppm	Crumb	Crumb	Crumb
C4 (2.4D 1 + BAP 0.2) ppm	-	-	-
C5 (2.4D 1.5 + BAP 0.2)	Crumb	Crumb	Compact

ppm			
C6 (2.4D 2 + BAP 0.2) ppm	Crumb	Crumb	Crumb

The results of the callus culture observation on leaf explants of *C. arabica* L. got various callus results. Figure 1 days of callus emergence after planting for the 6 treatments tested, the best callus percentage was in treatment C6 because C6 (2.4 D ppm + BAP 0.2 ppm) produced callus with an average of 26.67 days. Meanwhile, the percentage of callus in treatment C2 and C4 showed no callus growth. In this study, the average callus formed was only found in explant incisions, in contrast to the results of Priyono's (1993) study which found callus and somatic embryos on the surface and leaf sides. But this study found the same case in the results of research Oktavia *et al*., (2003) that callus only formed in the explant incision.

Figure 1 Observation of callus culture from leaf explants of *C. arabica* L. Treatment C1, C3, C5, C6 got a response to callus appearance, while in treatments C2 and C4 swelling occurred, not showing callus appearance. Ajijah *et al*., (2010) stated that swelling in explants is an early stage of callus formation which indicates cell activity in explants. The swelling response occurs because of the interaction between the explants and the growth environment and growth regulators through the absorption of nutrients by the explants.

According to Sitinjak *et al*., (2015).enlargement of cells that occurs as a result of cell activity in the form of absorption of water and nutrients which results in explants are swollen. In this study, explants that experienced swelling experienced a change in color from green to brown (browning) so that the growth of callus was inhibited. Sitinjak (2015), also stated that the wounding process given to explants was thought to be a factor that affected the explants to brown. This damage is caused by phenolic compounds that accumulate in cells and then undergo oxidation.

Callus that were successfully induced from several treatments had different color responses. Color can be an indicator of callus representation that still has active cells that are dividing or are heading to the death stage. The color of the callus can be seen in (Table 1) the white callus is still actively dividing compared to the brown callus that has died so that no development occurs (Hayati *et al*, 2010).

Callus with white color is considered good because it has a high content of starch grains. White callus is embryonic tissue that does not contain chloroplasts, but has a high starch content (Tsuro, 1998). In addition, Fatmawati (2008), stated that callus that is white or bright means that the callus growth is in quite good condition. The color change that occurs is due to the pigmentation of the chlorophyll which is degraded.

In a study with 6 treatments, the callus formed had a crumbly and compact texture. This depended on the development of the explants. Crumb callus is characterized by the structure of the cells being tenuous and easily brittle, compact callus has a tight cell arrangement and forms a solid bulge, while for intermediate callus a combination of compact callus and crumbs. Callus texture can be seen in (Table 2).

Good callus is a callus that has a crumb texture because it is easily separated into single cells (Armila *et al*., 2014). Compact type callus generally has slow growth, is difficult to separate and looks solid but compact type callus is considered good for use as a producer of secondary metabolites (Indah *et al*., 2013).

IV. CONCLUSION

The conclusion that can be obtained from this study is that the concentration in the treatment media of the best combination of auxin and cytokinin for callus induction of Coffea arabica L. in treatment C6 (2.4 D 2 ppm + BAP 0.2 ppm) with an average callus appearance of 26.67 days after planting, the texture of callus crumbs and callus is white, which means that it is still active in cell division.

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