Embryogenic Callus Induction of Todolo Toraja Coffee Leaf Cells (Coffea arabica Var. Typica) with the Addition Of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Furfurylaminopurine (Kinetin) in Vitro

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Abstract. Somatic embryogenesis is a way to produce large numbers of seeds according to the parent, so it is very profitable in the effort to conserve Arabica coffee, which has reduced number of trees and is not productive. This study uses Todolo arabica coffee (Coffea arabica Var. Typica) which is the oldest commercial coffee found in Toraja, South Sulawesi and is threatened with extinction. This study aims to obtain the best composition in administering zpt 2,4-D and kinetin in forming embryogenic callus from explants of arabica coffee leaves. This research was conducted at the Tissue Culture Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University. The experimental range used was a completely randomized design with 2 factorials, namely the concentration of 2,4-D 1 ppm, 2 ppm, and 3 ppm and kinetin concentration of 0.1 ppm, 0.5 ppm and 1.0 ppm so that there were 9 treatments with 3 replications. The parameters observed were callus formation time, callus structure, and callus color. The results showed that the fastest time for callus appearance was 17.00 HSK with 1 ppm 2,4-D and 0.5 ppm kinetin. The callus texture formed is a crumb-textured callus with several color variations that are formed.

1. Introduction

Coffee is one of the plantation commodities that has a high selling value among other plantation crops. Apart from that, it is also a huge source of foreign exchange for the country. Indonesia is the third largest coffee producing country in the world after Brazil and Vietnam. There are two types of coffee developed in Indonesia, namely Arabica coffee and Robusta coffee [1]. Arabica coffee is a traditional coffee that is considered the most delicious.

South Sulawesi is one of the provinces in Eastern Indonesia which has the potential for developing coffee. This is indicated by the planting area which is quite large and supporting agro-climatology [2]. One of the areas that are famous for producing arabica coffee is Enrekang and Toraja, even in the Toraja forest area there are also still found varieties of typica arabica coffee which is the oldest commercial coffee which is better known by the local population as Todolo coffee. However, the existence of this coffee is threatened with extinction, there are only a few trees and it is hundreds of years old so it is no longer productive.

Arabica coffee propagation can be done generatively using seeds or vegetatively using cuttings, grafting, and shoot grafting. However, this propagation has several weaknesses including limitations on
the amount of planting material as seeds and also propagation using seeds does not guarantee that the seeds produced will be the same as their parents [3] [4].

Typika arabica coffee is very susceptible to leaf rust disease (Hemileia vastatrix), and arabica coffee found in the Toraja area is hundreds of years old so it is no longer productive. Its habitat has also been damaged due to land clearing and the number of local people who have crossed typica coffee with other types of arabica coffee, making this old coffee rare [5].

One of the tissue culture techniques is somatic embryogenesis culture which is the simplest and easiest method to be implemented in various plant breeding programs. Propagation through somatic embryogenesis has been carried out with various types of explants that have been used, including culture of anther, meristem, seeds, roots and leaves. According to the results of the study [6] that the use of coffee leaf explants was the most responsive in producing somatic embryos compared to other plant parts. In addition, the use of leaf explants in arabica coffee in the somatic embryogenesis process has been widely used, including [7] [8] [9].

Therefore, based on the description from the above background, research is needed to find the best combination of 2,4-D and kinetin to induce embryogenic callus from Todolo toraja arabica coffee leaf explants (Coffea Arabica var. Typika).

2. Materials and Methods

This research was conducted from June to August 2020 at the Tissue Culture Laboratory, FMIPA Hasanuddin University. The explants used were Arabica coffee leaves var. Typika in the second or third position from the top of the plant. The medium used was MS media (Murashige and Skoog) with ½ micro and macro salt concentrations equipped with vitamin B5 and a combination of growth regulators 2,4-D with a concentration of 1 ppm, 2 ppm, and 3 ppm and kinetin with a concentration of 0.1 ppm, 0.5 ppm and 1.0 ppm.

a. Sample preparation
The samples of young leaves of Arabica coffee which are in the second to third positions from the top of the plants that have been taken are then washed under running water and stored in a clean place and at room temperature.

b. Sterilization tool
All tools to be used are sterilized using autoclave at a temperature of 121°C for 15 minutes with a pressure of 2 atm. All tools to be used are entered into an autoclave.

c. Preparation of 2,4-D and Kinetin stock solutions
Each stock solution will be made with a concentration of 100 ppm. The hormone is weighed as much as 10 mg and will be dissolved in 100 ml of distilled water. The weighed hormone is put in a beaker and 70 ml of distilled water is added. After dissolving, add distilled water to 100 ml then put the stock solution into a glass bottle then cover with aluminum foil and store it in the refrigerator.

d. Making a culture medium
The medium used was synthetic ½ MS (Murashige & Skoog) medium. MS medium was weighed as much as 2.215 g for 1 liter of medium. Then add 30 gr / l of sucrose and 8 g / l of agar. Furthermore, the medium will be added to the growth regulator 2,4-D and kinetin with various treatment combinations. Furthermore, the media was sterilized using an autoclave at a temperature of 121°C for 15 minutes with a pressure of 2 atm.

e. Embryogenic Callus Induction
The leaves that have been cleaned with running water are then sterilized using a 0.2% solution of Dithane M-45 for 30 minutes, then rinsed thoroughly using sterile water. Then in laminar air flow, the leaves are
soaked in 70% alcohol for 3-5 minutes and followed by immersion in a 20% calcium hypochlorite solution for 15 minutes and shake every 5 minutes for 5 seconds. And then rinsed thoroughly using sterile water 3 times. The leaves were then cut into pieces ± 1 cm × 1 cm in size and then the explants were planted on the culture media. The cultures were incubated in a dark room at a temperature of 24 oC and a relative humidity of ± 60% for 2 months [10].

f. Data analysis
The data obtained from this study were then analyzed using descriptive analysis and inferential statistical analysis in the form F α 0.05 test used SPSS 22 software and continued with Duncan Multiple Range Test (DMRT) at a test level of 5%.

3. Result and Discussion
Somatic embryogenesis was initiated by inducing embryogenic callus using a combination of 2,4-D and kinetin. In this study, the explants used were young leaves of Arabica Todolo toraja coffee grown for 2 months on ½ MS medium. The addition of growth regulators in the form of 2,4-D and kinetin can induce explants to produce embryogenic callus. Callus formation begins in the second to third week after planting. The results showed that the fastest callus growing time was the treatment with the addition of 1 ppm 2,4-D and 0.5 ppm kinetin, namely 17.00 HST. The results data for callus growth days after planting can be seen in Figure 1.

All treatments showed the ability to induce callus, it can be seen in Figure 1 that all treatments based on the DMRT test showed significant differences to the control. This is because the presence of growth regulators is very important in inducing callus, so that the control without addition of 2,4-D or kinetin could not produce callus up to 2 observations (Figure 2A). Whereas the addition of growth regulators in treatment A2 (Figure 2B), namely the addition of 1 ppm 2,4-D and 0.5 ppm kinetin based on the DMRT test showed different real against all treatments.

Figure 1. Average days after planting callus. (A0) control, (A1) 1 ppm 2,4-D + 0.1 ppm kinetin, (A2) 1 ppm 2,4-D + 0.5 ppm kinetin, (A3) 1 ppm 2,4-D + 1 ppm kinetin, (A4) 2 ppm 2,4-D + 0.1 ppm kinetin, (A5) 2 ppm 2,4-D + 0.5 ppm kinetin, (A6) 2 ppm 2,4-D + 1 ppm kinetin, (A7) 3 ppm 2,4-D + 0.1 ppm kinetin, (A8) 3 ppm 2,4-D + 0.5 ppm kinetin, (A9) 3 ppm 2,4-D + 1 ppm kinetin. The letters were not significantly different in the 5% DMRT test.
This shows that the addition of 2,4-D and kinetin is able to induce callus on Todolo Toraja arabica coffee explants. The use of a combination of 2,4-D and kinetin has also been carried out to induce callus in robusta coffee [11]. This is in line with [10] who stated that the development of callus from explants and until callus was formed showed that the induction medium contained growth regulators affected callus growth. The administration of auxins is very effective in inducing callus formation, however, cytokinins have a necessary role in callus proliferation so that the combination of auxins and cytokinins is needed to stimulate callus growth [12].

Callus formation started from the edge of the explant of the incision area (Figure 2B) characterized by swelling and curved explants and the appearance of small white spots. According to [13], callus will appear on the cut explants in the form of small spots and will develop into clear and large spheres. The same thing was also found in the study of where callus grew on the incision scar. Injuries to cells or tissues will activate self-defense mechanisms that induce the expression of certain genes. In damaged tissue, an irregular and differentiated cell structure will form a callus. Figure 1 shows that the administration of 2,4-D 1 ppm and 0.5 ppm of kinetin can form callus faster than other treatments. It is thought that the higher the concentration of 2,4-D could inhibit callus growth on explants. The same thing was also found in the research of [14] showed that the average time when callus appeared slower the higher the 2,4-D concentration.

Based on morphological observations, the callus produced in this study is a crumb structure and is also intermediate (Figure 3). Crumb callus is a characteristic of embryogenic callus which will later develop into somatic callus. According to [15], callus texture is divided into three, namely compact, intermediate and crumb callus. The crumbly callus makes it easy to multiply the number of calluses. Calluses that are yellowish in color and yellowish white are embryogenic calluses, while white calluses are non-embryogenic calluses that will not develop into somatic calluses [10].
In the study, there were several calluses that were brownish in color. This is thought to be due to the toxic metabolism of phenol. Phenolic compounds will be oxidized to from quinones which have toxic properties to plant cell and can cause explant death [16].

4. Conclusion
Giving a combination of 2,4-D and kinein against the growth of embryogenik callus on leaf explants of Arabica coffee var typica can form embryogenik callus in all treatments. For the best combion, the administration of 1 ppm 2,4-D and 0.5 ppm of kinetin that can produce the fastest callus and produce crumb callus which is a characteristic of embryogenic callus.

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