SOMATIC EMBRYOGENESIS OF ARABICA COFFEE (*Coffea arabica* var. *Lini-s* 795) FROM TORAJA BY *IN VITRO* WITH THE ADDITIONAL OF 2,4 DICHLOROPHENOXYACETID ACID (2,4 D) AND 6 FURFURYLAMINO PURINE (KINETIN)

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ABSTRACT

In general, coffee propagation still uses seeds, cuttings, grafting and shoot grafting. However, this method of propagation still has limitations in the amount of planting material. With somatic embryogenesis, it is possible to produce relatively uniform seedlings on a large scale and in a relatively short time. This study analyzed the effect of providing 2,4-dichlorophenoxyacetic acid (2,4 D) and 6-furfuryl-aminopurine (kinetin) in the process of forming embryogenic callus and somatic embryos from var.lini-s 795 arabica coffee leaves. This research was conducted at the Tissue Culture Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University. The plant materiala used were var.lini-s 795 arabica coffee leaves. This study used 9 treatments and 1 control and used a completely randomized design with 3 replications. The parameters observed were the speed of the explants forming callus, the percentage of embryogenic callus, callus color, callus texture, callus wet weight, and the total of globules. The observation showed that all treatments were successful in forming callus except the control treatment. T he fastest treatment to produce callus was 2 ppm 2.4D and 2 ppm kinetin. The highest wet weight of callus was obtained in the treatment of 2.4 D 0.5 ppm and kinetin 0.5 ppm with a weight of 1.46 grams, the most embryogenic callus was obtained in treatment of 0.5 ppm 2.4 D and kinetin 0.5 ppm with a percentage 98% and the most somatic embryos in the form of golbular were obtained in the treatment of 0.5 ppm 2.4D and 2 ppm kinetin, which was 68.33.

Keywords: *Coffea arabika*, 2,4 D, kinetin, somatic embryo

INTRODUCTION

Arabica coffee (*Coffea arabica L.*) is a plantation crop that has been cultivated and has an important role in the national economy. Propagation of arabica coffee is generally done generatively using seeds or vegetatively using cuttings, grafting, and shoot grafting. However, this method of propagation still has limitations in the amount of planting material. By using tissue culture techniques, it can provide an alternative in coffee propagation. This technique makes it possible to produce relatively uniform seedlings on a large scale with the same plant quality as the parent in a relatively short time, and free from pests and diseases (Pinto et al., 2019). Various approaches have been considered for the propagation of coffee tissue cultures including, organogenesis, (using adventitious and axillary shoots), and somatic embryogenesis (Andres et al.,2008).

Propagation through somatic embryogenesis can be done using culture of anther, meristem, seeds, hypocotyl, epicotyl, root and leaf. The results of the study using leaf explants in arabica coffee in the somatic embryogenesis process were the most responsive in producing

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somatic embryos compared to other plant parts . (Figueroa-Quiroz et al. 2002; Albarra´n et al., 2005; Ahmed et al., 2013; Ibrahim et al., 2013; Kahia et al., 2016).

In addition to the use of appropriate explants, basic media and growth regulators are needed in the propagation of Arabica coffee through somatic embryogenesis. An important factor in the induction and development of somatic cell embryogenesis is the nutritional composition of the culture medium (Santos-Briones and Hernández-Sotomayor, 2006; Méndez-Hernández et al., 2019). This study aims to determine the effect of 2,4-dichlorophenoxyacetic acid and 6-furfuryl-aminopurine in the induction of embryogenic callus to somatic embryos.

MATERIAL AND METHOD

The explants used were young leaves that had opened perfectly in the second and third positions of var.lini-s 795 arabica coffee shoots. The basic medium used was ½ concentration of MS medium (Murashige & Skoog), vitamin B5, 30 g/l sucrose, polyvynil pyrolidone (PVP), a *6-Furfuryl amino purine* (Kinetin) growth regulator (0.5, 1, 2, ppm) and 2,4 *Dichlorophenoxy acetic acid* (2,4 D) (0,5, 1, 2, ppm). 0.5 ppm 2,4-D + kinetin 0.5 ppm, 0.5 ppm 2,4-D + 1 ppm kinetin, 0.5 ppm 2,4-D + 2 ppm kinetin, 1 ppm 2,4-D + 0.5 ppm kinetin, 1 ppm 2,4-D + 1 ppm kinetin, 2,4-D 1 ppm + 2 ppm kinetin, 2 ppm 2,4-D + 0.5 ppm kinetin, 2 ppm 2,4-D + 1 ppm kinetin, 2 ppm 2,4-D + 2 ppm kinetin, with 3 repetitions. The pH of the media was adjusted to 5.7. Furthermore, the media was sterilized by autoclaving at a temperature of 121^0c for 30 minutes with a pressure of 1.5 atm.

Induction of Embryogenic Callus and Somatic Embryos

The leaves that had been cleaned with running water were soaked in a fungicide solution of 0.2% of Dithane M-45 for 1 hour, then rinsed thoroughly. Sterilization was carried out using 70% alcohol for 3 minutes and 10% sodium hypochlorite for 10 minutes, then the leaves were rinsed thoroughly using sterile aquadest. The sterile leaves were cut into pieces ± 1 cm x 1 cm in size and grown on culture media. All sterilization and explant planting were carried out in laminar air flow.

Culture bottles containing explants were incubated in a dark room at a temperature of ± 25°C with a relative humidity of ± 60% for 2 months. After 2 months, embryogenic callus were formed, then subcultured on the same medium to produce somatic embryos. The parameters observed were the rate of callus forming explants, the percentage of embryogenic callus, callus color, callus texture, callus wet weight, and globular formed.

Statistical Analysis

The data obtained from this study were analyzed with the SPSS 24 program. If there is a significant difference, then a further test is carried out with the Duncan Multiple Range Test (DMRT) at the test level of 5%.

RESULTS AND DISCUSSION

Induction of Embryogenic Callus and Somatic Embryos

The results of observations of leaf explants began to swell in the first week and the second week of callus was formed on the incision marks in the combination medium of 2 ppm 2,4-D + 2 ppm kinetin and in other treatments there was scaling in the following week except in the control treatment (without giving 2, 4 D and kinetin). The callus was seen growing after 2 months in the media and had a crumb structure. The stages of change from callus to forming globular somatic embryos can be seen in Figure 1. The length of time required for explants to form callus is different, each genotype and plant tissue contains different endogenous growth

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regulators so that the tissue's ability to absorb nutrients in the media and the resulting response is also different (Loyola-Vargas et al 2016).

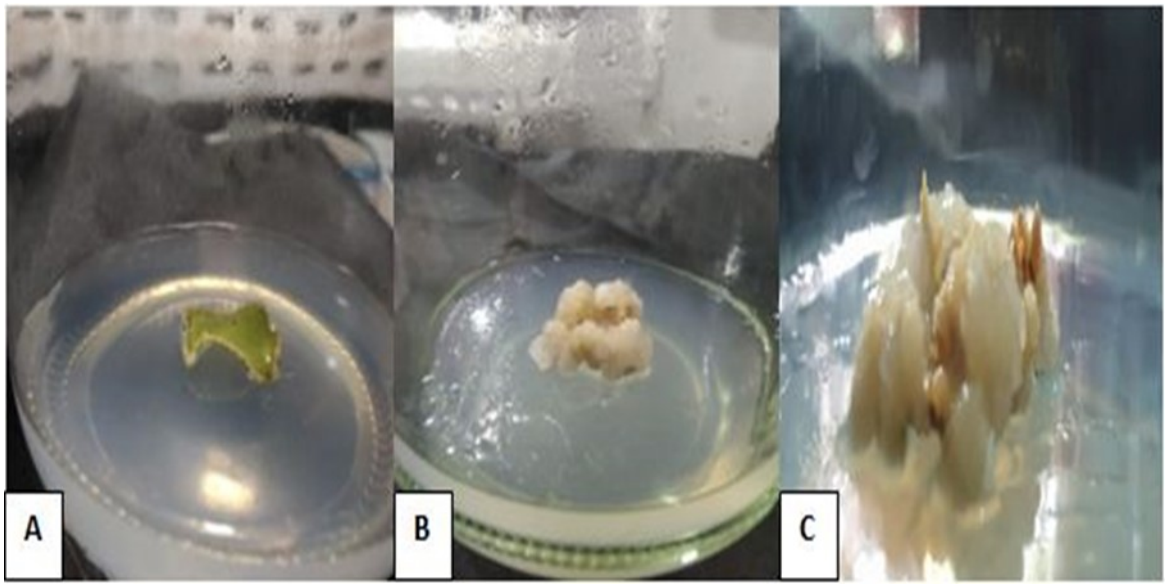

Figure. 1 Callus development performance through the embryogenesis process in induction media. A) Callus began to form in the second week, B) Embryogenic callus formed 2 months after culture, C) Somatic embryos were formed.

Observations were made based on the morphology of the callus that was formed, not all of them were embryogenic, there were still non-embryogenic calluses among the embryogenic calluses. The average percentage of embryogenic callus can be seen in (Figure 2). Non-embryogenic callus is a callus that does not have the potential to regenerate into plantlets. The percentage of embryogenic callus is influenced by the high and low concentration of the synthetic auxin hormone which functions to induce embryogenic callus (Chithra et al. 2005). In the induction phase, the explants are stimulated to form embryogenic callus, namely callus which consists of cells that have the ability to form somatic embryos that are ready to germinate (Arnold, 2008).

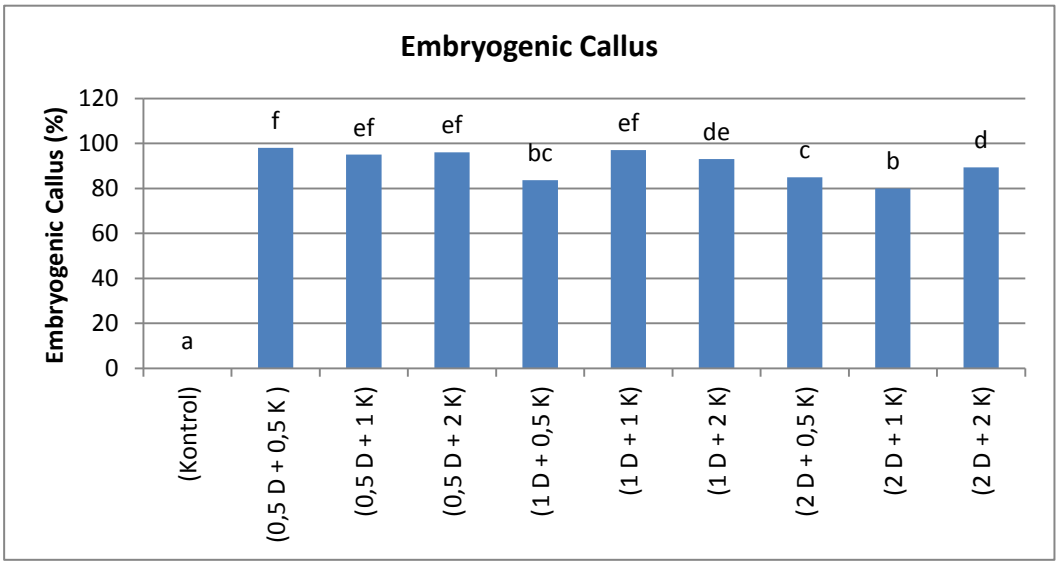

Figure 2. Percentage of embryogenic callus after 2 months of culture on callus induction media. The same letter is not significantly different based on the 5% level of DMRT.

The callus colors of arabica coffee var.lini-s 795 that are formed are grouped into four groups, namely yellowish white, brownish callus, brownish yellow callus, and white callus. Callus that is yellowish, yellowish-white and brownish in color is an embryogenic callus. Embryogenic callus has the potential to become somatic embryo callus whereas white callus is non-embryogenic callus and this callus does not have the potential to become somatic embryo callus (Quiroz-Figueroa et al., 2006).

Based on data on callus wet weight in the treatment of 0.5 ppm 2.4 D + 0.5 ppm Kinetin produced the best wet weight of callus of 1.46 grams. The data can be seen in Figure 3. This is presumably because the concentration of growth regulators in the media is able to induce callus growth well, so that callus growth is faster followed by an increase in callus volume and mass. The large fresh weight of the callus is due to its high water content. The resulting wet weight is highly dependent on the speed at which these cells divide, multiply and continue with the enlargement of the callus (Ariani, at al 2016).

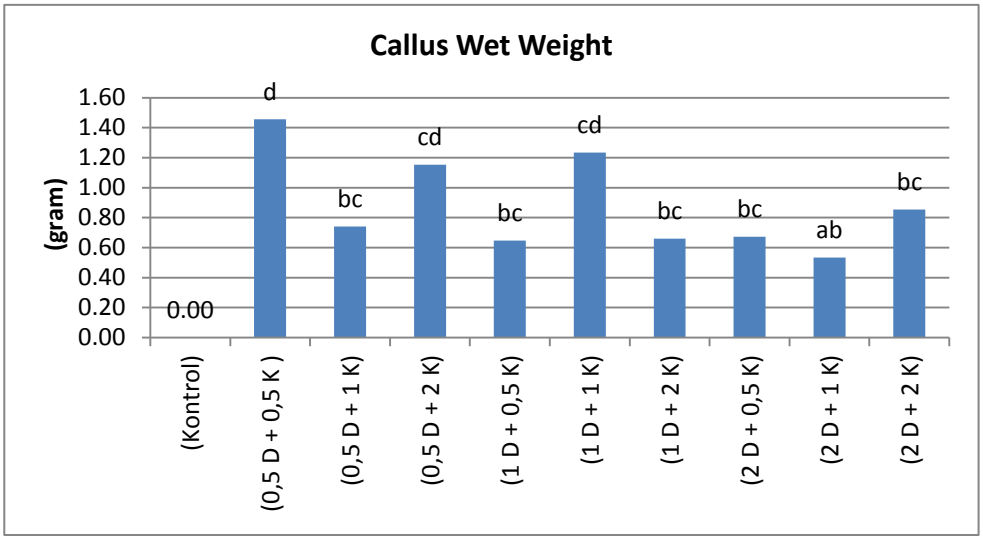

Figure 3.Callus wet weight after 2 months of culture on callus induction media. The same letter is not significantly different based on the 5% level of DMRT.

The results of the observations were that the sub cultured embryogenic callus succeeded in forming somatic embryos in all treatments. However, the numbers are still lacking. The treatment of 0.5 ppm 2.4 D and 0.5 ppm kinetin was the result of the most embryogenic induction but when it entered the somatic embryo stage it resulted in very small somatic embryos, namely 32. This is thought to have occurred because of the use of different combinations of auxin and cytokinins in all treatments. The induction of different somatic embryos can be caused by variations in auxin concentrations (Riyadi, A dan Tirtoboma.2004). The use of auxins and cytokinins can stimulate embryogenesis, but a certain ratio of the two combinations is required to induce somatic embryos (Samson et al., 2006; Mene´ndez-Yuffa´ et al. 2010). In this study, the best combination results that were able to induce the most somatic embryos were in the treatment of 0.5 ppm 2.4 D and 2 ppm kinetin, namely 68.33. Data can be seen in Figure 4. In coffee plants, propagation through somatic embryogenesis uses the following steps: primary callus induction, embryogenic callus induction, embryo regeneration or formation, and plant regeneration from embryos (Samson et al., 2006; Ducos et al., 2007).

The beginning of the somatic embryogenesis process begins with the formation of rounded projections, which usually arise from explants or calluses. The development of normal somatic embryogenesis will go through stages, globular, heart shape, torpedoes, cotyledons.

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After the somatic embryo reaches the cotyledons, shoots begin to form and reach the stage of becoming a new plant or plantlet (Yang and zhang, 2010; Sanglard et al., 2019).

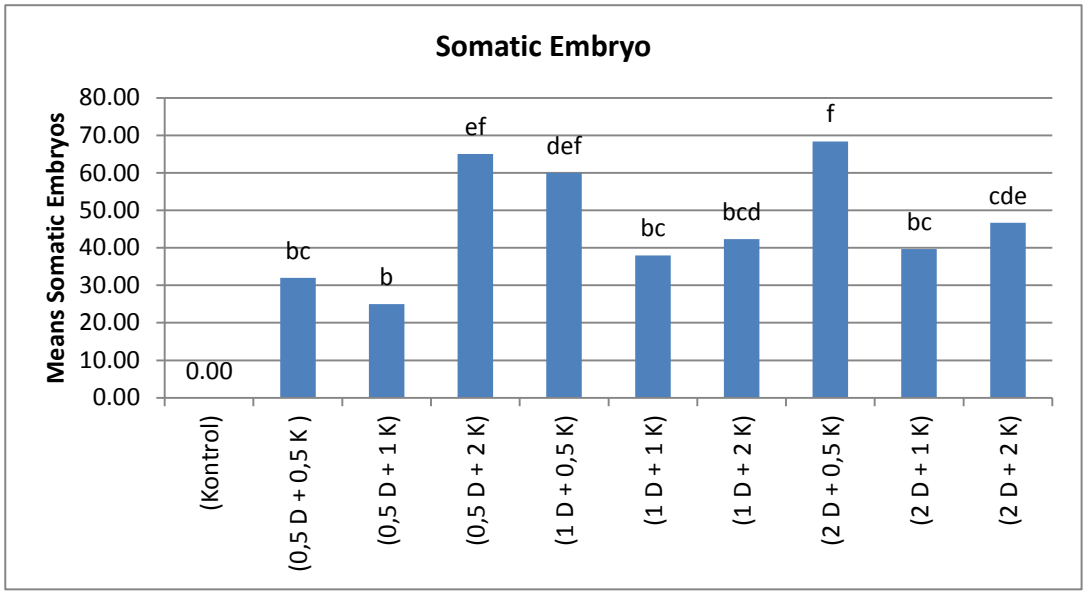

Figure 4. Graph of mean somatic embryo callus after subculture on the same induction medium for 2 months

CONCLUSION

Embryogenic callus was successfully induced from arabica coffee leaf explants in all treatments except the control treatment. The most embryogenic callus was produced in the treatment of 0.5 ppm 2.4 D + 0.5 ppm kinetin with a percentage of 98% and the least in the treatment of 1 ppm 2.4 D and 2 ppm kinetin resulted in embryogenic callus that was 80% and embryogenic callus which In subcultures, the most somatic embryos were produced in the treatment of 0.5 ppm 2.4 D and 2 ppm kinetin, namely 68.33 and the least in the treatment of 1 ppm 2.4 D and 0.5 ppm kinetin, namely 25.

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