Embryogenic callus induction of coffee \([\textit{Coffea arabica} \text{ L.}]\) on several plant growth regulator concentration and incubation temperature

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Abstract. Clonal propagation using in vitro techniques to provide good quality seedling of coffee requires technological establishment, especially about the determinant factor for regeneration success. Therefore the study of regeneration methods in vitro in coffee plants needs to be done either through somatic embryogenesis or organogenesis. The purpose of this research was to obtain the optimal concentration of plant growth regulators and incubation temperatures to induce the embryogenic callus of coffee. The basic medium was used MS [Murasige and Skoog] with the addition of 1 g/L of active charcoal and 7 g/L of bactoagar. The experiment used a factorial completely randomized design. The first factor is the concentration of plant growth regulators consisting of 6 levels: 0.5 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\); 0.5 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\); 1 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\); 1 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\); 2 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\); 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\). The second factor is the incubation of temperature is consisting of 2 levels: 18°C and 26°C. Variables observed included: time of callus induction, percentage of callus induction, callus texture and callus color. The results showed that all medium tested only media with a concentration of 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) which gave an optimal response to the percentage of callus induction which reached 100% with friable texture and yellowish-white. Based on observations it is also known that the incubation temperature of 26°C can induce callus faster than the incubation temperature of 18°C.

Keywords: callus, coffee, organogenesis, regeneration, somatic embryogenesis

1. Introduction

Coffee \([\textit{Coffea} \text{ sp.}]\) is a plant species from family Rubiaceae and the genus Coffea. This plant is classified as a plantation that can grow and develop well at various altitudes. The level of coffee consumption per capita of the Indonesian is lower than the European community who consume an average of coffee above 5 kg/capita/year while the United States is above 4 kg/capita/year. Along with the development of technology and industry as well as the high demand for global coffee consumption, the trend is increasing, the national coffee production needs to get a boost in increasing production \([1]\).

The low productivity of coffee is partly because 95% of Indonesian coffee is smallholder estate. The smallholder estate productivity is still low, which is only 50-60% of the potential production. One reason is that farmers generally do not use good quality of seedling. Besides the cultivation technique applied still simple so that maintenance is not intensive, the slow rejuvenation of plants, and the lack
of supporting facilities and infrastructure. As a result of these conditions resulted in poor quality coffee [1].

To offset the ever-increasing demand for coffee, there is no other way to do this than through increasing production. To achieve this, the availability of quality seedling is the most important requirement. Propagation of coffee is generally done by generative means through seeds. One disadvantage method is that the morphology of the puppies obtained is not uniform but also has quite a big difference from its parent. Tissue culture is a plant propagation technique that can be used to produce planting material in large quantities in a relatively short time. Besides is tissue culture also very necessary in plant breeding programs to get superior seeds, pest-free disease, and high productivity [2].

Breeding in tissue culture can be done through organogenesis and somatic embryogenesis. Propagation of coffee through somatic embryogenesis from various types of explant sources has been carried out using anther, root, seed, leaf, epicotyl, hypocotyl, and meristem culture. The results showed that the use of coffee leaf explants was the most responsive in producing somatic embryos compared to other plant parts [3]. Somatic embryogenesis is a more appropriate propagation technique for coffee because it allows large-scale clonal propagation with relatively lower production costs [4].

Propagation of Arabica coffee through somatic embryogenesis techniques is an important part of providing large quantities of seedlings. The somatic embryogenesis techniques also the basis for the use of biotechnology applications in increasing genetic diversity such as mutation, in vitro breeding and genetic engineering.

2. Material and Methods
This research was conducted in May - September 2019 at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University. Sigararutang and Sinar Harapan genotype used as explants, MS media [Murashige and Skoog], sucrose, vitamins, myoinositol, 2,4-D growth regulators, TDZ, bacto agar, bactericide [agrept], fungicide [dithane], HCl 0.1 N, PVP, 0.1N KOH, 70% alcohol, 96% alcohol, tween 20 solution, sterile aqua dest, bayclean, heat-resistant clear plastic, plastic wrap, black plastic, masking tape, label paper, aluminum foil, tissue, rubber, spritus, HVS detergent, and paper. The basic medium used for callus induction was 1/2 the concentration of MS and micro salt [Murashige and Skoog] supplemented with vitamin B5, 30 g L-1 sucrose, and 250 mg L-1 Polyviynyl polypyridine [PVP]. Bacto agar is added to 8 g L-1.

The study was arranged based on a Factorial completely randomized design. The treatments tested are: 0.5 mg 2.4D L-1 + 1 mg TDZ L-1; 0.5 mg 2.4D L-1 + 2 mg TDZ L-1; 1 mg 2.4D L-1 + 1 mg TDZ L-1; 1 mg 2.4D L-1 + 2 mg TDZ L-1; 2 mg 2.4D L-1 + 1 mg TDZ L-1; 2 mg 2.4D L-1 + 2 mg TDZ L-1. The culture was incubated in a dark room at ± 18oC and ± 26oC for 2 months. Variables observed were: Time of callus induction, callus induction percentage, callus texture and callus colour.

3. Result and Discussion
3.1. Time of callus induction [day]
The emergence of callus begins with the initiation of the leaf pieces. Callus size increases with incubation time. media with the addition of 2 mg 2.4D L-1 + 2 mg TDZ L-1 were able to induce callus most quickly, which was about 9 days after culture in the Sigarar Utang variety and 13.5 days after culture in the Sinar Harapan genotype [Figure 1].

Plant Growth regulator [PGR] with low concentrations are unable to induce callus formation. One month in the induction media, the callus that formed only around the former explant pieces then continued to expand and to cover all surfaces of explants [Figure 2]. This was also found in the study of Oktaviana et al. [3] where the callus was only formed by a cut in explants. This is different from the results of research by Priyono [5] which succeeded in getting somatic callus and embryos on the surface or sides of the leaf. This difference is thought to be due to differences in explant size used, different concentrations and types of PGR added to the callus initiation media, duration of initiation,
as well as differences in response from plant genotypes used so that tissue responsiveness is also
different.

![Figure 1](image1.png)

**Figure 1.** Time of callus induction with different concentrations of plant growth regulators and incubation temperature. A] Sigarar Utang, B] Sinar Harapan.

![Figure 2](image2.png)

**Figure 2.** Development callus of arabica coffee. A] young leaf explants 1 week after culture [wac], b] 3 wac, c] 4 wac, d] 5 wac, e] 6 wac, f] 7 wac.

PGR is very important in the formation of callus. That was reported by Ibrahim et al. [6], 2,4-D and kinetin in arabica coffee were able to produce callus. They have reported that auxin and cytokines given together can stimulate the formation of embryogenic callus, but certain ratios of the combination of both are needed to induce somatic embryo formation. This is consistent with previous research which reported that when arabica coffee explant tissue was placed in a nutrient medium containing auxin and cytokinin growth regulators, somatic cells carried out the process of differentiation to form a callus and pro-embryonic masses [7] [8]. Incubation temperature also has influenced the time of the callus. Incubation at 26°C can accelerate the emergence of callus in coffee plants. This is thought to be caused by the increased rate of cell metabolism when incubated at high temperatures compared to low temperatures.

### 3.2. Percentage of callus induction.

Based on observations that have been made on the percentage of callus induction showed that from the six media used only the media with the addition of 2 mg 2,4D L⁻¹ + 2 mg TDZ L⁻¹ which gave the best effect with the callus induction percentage reaching 100% at both incubation temperatures [Figure 3]. Explant initiation occurs in the one week after culture. After the explants swell, the callus begins to appear at the edge of the explant. A callus is a group of cells that have not been differentiated and are actively dividing to form an unorganized cell mass [9]. A callus that begins to grow on the explant
continues to grow until it covers the entire surface of the explant. Basically, every cell has the nature of the totipotence of cells, thus forming a new individual if the environmental conditions are appropriate [10]. In the formation of callus several factors that influence, such as the type of explant, medium, incubation space conditions, and plant growth regulators.

Based on observations, the 2,4-D and TDZ are capable of producing 100% callus induction. This is supported by Arianto et al. [11] reported 2,4-D is a strong auxin that plays a role in cell division, thus supporting the formation of cocoa callus. PGR 2,4-D is more effective combined with cytokines. This is also the following research conducted by Riyadi and Tirtoboma [12] in inducing somatic of arabica coffee embryos, all 2,4-D treatments combined with kinetin were able to produce somatic embryos in different percentages.

3.3. Callus Texture

The results of coffee texture observations visually showed that all treatments tested had a friable textured callus. Friable textured callus can be observed visually. The friable textured callus looks like grains that are easily separated in all parts. Callus texture, in general, can be grouped into two, namely friable textured callus and compact textured callus. A friable-textured callus is a callus that has a large inter-cell space and tenuous cell-to-cell bonds, so that the callus breaks easily when separated, whereas a compact-textured callus has a dense cell-to-cell bond so that the space between cells is not visible and the particles callus particles are not easily separated. Zulfitra [13] has induced coffee plants and produced friable-textured callus with the characteristics of clear and yellowish-white callus that has the potential to form higher embryos than the compact and blackish-brown callus.

Table 1. Callus texture of coffee plants at different concentrations of growth regulators and incubation temperatures.

| Medium                              | Sigarar Utang | Sinar Harapan |
|-------------------------------------|---------------|---------------|
|                                     | 18°C          | 26°C          | 18°C          | 26°C          |
| 0.5 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\) | -             | -             | -             | -             |
| 0.5 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) | -             | -             | -             | -             |
| 1 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\)   | -             | -             | -             | -             |
| 1 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\)   | -             | Friable       | -             | -             |
| 2 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\)   | -             | Friable       | -             | -             |
| 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\)   | Friable       | Friable       | Friable       | Friable       |

The texture of the callus also determines the direction of the development of the callus itself. Trisnia [14] states that callus consists of two types, namely embryogenic and organogenesis. An
embryogenic callus is a callus that has a friable texture and has a yellowish-white, yellow, and brownish yellow color. Whereas the callus which has a compact texture and white color, the compact callus indicates that the callus is organogenesis. However, in the study of Ajizah et al. [15] embryogenic callus produced by several cocoa clones has a friable texture with a creamy brownish and yellowish tint using a growth regulator TDZ. Widyawati and Geningsih [16] stated that callus with friable texture could be caused by the presence of endogenous growth-regulating agents in the explants, adding 2,4-D to the media would accelerate callus formation because 2,4-D was able to stimulate cell division and enlargement, so will spur callus formation and growth to be faster. In addition to 2,4-D callus with friable texture is also influenced by the addition of cytokines in the media, friable textured callus experiences cell division faster than compact textured callus [17].

The ability of 2,4-D to form callus with friable texture supported by Nisak et al. [18] reported that 2,4D can stimulate cell elongation by adding cell wall plasticity to become loose, so that water can enter the cell wall through osmosis and cells are elongated, therefore the friable callus contains a lot of water because have not undergone cell wall lignification, and between a collection of cells with one another is relatively easy to separate. Based on observations obtained different callus color results. The white callus was the most dominant in the treatment tested except in the treatment of 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) which was dominated by a yellowish-white callus. The higher the concentration of 2,4D due to the percentage of callus with white color decreases. The color of the callus was observed from the first time the callus appeared on the surface of explants in the two weeks after culture. The callus that appears first is white and then changes color in several explants, but there is also callus that does not change color until the end of the observation. At the end of the observation, the callus produced two colors namely yellowish white and white.

**Table 2.** Callus color of coffee plants at different concentrations of growth regulators and incubation temperatures

| Medium                      | Sigar Utang 18°C | Sigar Utang 26°C | Sinar Harapan 18°C | Sinar Harapan 26°C |
|-----------------------------|------------------|------------------|--------------------|--------------------|
| 0.5 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\) | -                | -                | -                  | -                  |
| 0.5 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) | -                | -                | -                  | -                  |
| 1 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\) | -                | -                | -                  | -                  |
| 1 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) | -                | white            | -                  | -                  |
| 2 mg 2.4D L\(^{-1}\) + 1 mg TDZ L\(^{-1}\) | -                | white            | -                  | -                  |
| 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) | yellowish-white | yellowish-white | yellowish-white    | yellowish-white    |

4. **Conclusion**

The results showed that all medium tested only media with a concentration of 2 mg 2.4D L\(^{-1}\) + 2 mg TDZ L\(^{-1}\) which gave an optimal response to the percentage of callus induction which reached 100% with friable texture and yellowish-white. Based on observations it is also known that the incubation temperature of 26°C can induce callus faster than the incubation temperature of 18°C.

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