Propagation of Gembili (*Dioscorea esculenta*. L) Accession from West Papua by In-Vitro Callus Induction

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Abstract. Diversification of tuber foods is expected to help food security and improve public health. Gembili tuber glucomannan contains high fiber. Gembili seeds are becoming scarce, causing gembili plants to be very rarely found in the community. In the West Papua region, local people still rely on conventional supplies which are feared to threaten the gembili plant population. Tissue Culture Technology is very necessary in multiplying gembili seeds quickly and without knowing the season, by growing callus, plant propagation does not require a lot of explants to produce a large number of seeds. The research was conducted at the Politeknik Negeri Lampung Tissue Culture Laboratory, from May to July 2022. The research used a randomized block design, with each treatment repeated three times. The results showed that the composition of the media with the addition of 2.4 D 2 $mL.L^{-1}$ produces an average of 8.00 days of callus growth, however, for callus growth the culture media with the addition of 2.4 D $mL.L^{-1}$ produces a value best. 1.73 cm for callus diameter and a value of 0.22 g for callus weight.

1 Introduction

The contribution of tuber plants as an alternative food source has quite a big influence on food security. Tubers are food that is safe for public consumption, especially for people with diabetes and obesity. On the other hand, currently rice is still a staple food with quite high levels of participation in various regions, including in regions that previously had a non-rice staple food pattern. In fact, in several provinces there has been a shift in staple foods from various patterns to a single pattern, such as rice. Local foods such as corn, sweet potatoes and cassava are increasingly being abandoned by people [1].

One type of tuber that can be used in food diversification is gembili tubers. Papuan people have been cultivating gembili tubers for a long time, but not yet on a mass scale. The people of West Papua still rely on the availability of gembili tubers from nature, they are used to planting gembili tubers in the forest without proper cultivation [2]. The concern of countries around the world is about food security and nutrition in a sustainable food system. In dealing with food insecurity, it is very important to diversify the agricultural system by looking for

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alternative food ingredients with high carbohydrate value. Several root crops and wild tubers play an important role in food security programs in developing countries. Sweet potatoes (*Dioscorea spp*.) can be used as food and medicine for people who live mainly in tropical and sub-tropical areas. This plant is known as the fourth most important root crop after potatoes, cassava, and sweet potatoes [3].

Gembili tuber consumption is still low because gembili tubers are rarely available on the market due to low production levels. The harvest period for gembili tubers is relatively long and the unavailability of seeds in large quantities for commercial cultivation also influences this. The problem in cultivating gembili is the lack of public knowledge about gembili plants and the lack of availability of seeds.

Gembili is part of the Dioscorea family and is a climbing plant with monocot tubers and high nutritional value. Conventional tuber propagation is limited by low levels of multiplication, therefore in-vitro propagation provides the best alternative to overcome this problem [4]. For germplasm conservation activities and plant propagation, in-vitro plant biotechnology techniques are still considered a powerful tool. Based on callus cultivation, an optimal regeneration procedure was developed for indirect budding [5].

Many endemic plants face extinction due to harvesting, limited distribution and damaged habitats, so the tissue culture method is a successful method for producing plant secondary metabolites [6]. Thus, the use of biotechnology can help multiply gembili plants en masse and quickly, including through the use of in vitro culture techniques. An efficient method of callus induction and gembili regeneration through in vitro culture is needed with limited explant resources. Callus induction is the initial stage of an in vitro culture technique which aims to produce and multiply callus cells en masse.

Shoot regeneration activities always begin with the growth of callus, the explants are cultured in callus-inducing media which is rich in auxin and then the resulting callus is cultured in shoot-inducing media which is rich in cytokinins [7]. With the large number of cells formed, a large number of plants will be produced using only a limited number of explants. The aim of this research is to analyze the effect of the interaction of BAP and 2,4 D on the ability to form callus from gembili tubers from various plant parts in vitro.

2. Materials and methods

2.1 Place and Time of Research

The research was carried out at the Tissue Culture Laboratory, Food Plant Cultivation Department, Politeknik Negeri Lampung from May to July 2022.

2.2 Experimental design and data analysis

The two treatments compared were (A) the concentration of the growth regulator (ZPT) used, namely BAP and 2.4 D, and (B) plant parts, namely segments and leaves as a source of explants. There were 16 treatment combinations with each treatment repeated three times.

Table 1. Results of proximate analysis of dissected *gembili* tubers from West Papua

Water (%)	Ash (%)	Fat (%)	Proteins (%)	Crude Fiber (%)	Carbohydrate (%)
76.0887	0.5510	0.4191	0.7613	1.2557	22.1800

Gembili tubers before being used for research activities, and tuber samples were analyzed proximately to determine the content of *gembili* tubers (Table 1). The variables observed in

this study included the speed of callus growth/days of callus appearance (days) and callus diameter. The collected data was analyzed using statistics 8.

3 Results and Discussion

3.1 Quantitative Observation of Callus Growth Speed (Days)

Callus is undifferentiated cells that form on one or all of the explant slices. In this study, callus was formed on the nodes, whereas the effect of PGR on callus growth was not visible on leaf explants. Callus formation is characterized by swelling on the part that directly touches the media. For glands that experience swelling and callus growth, the media is treated with 2.4 D (1 mL/L and 2 mL/L) to form a callus. Research on Nigella damascena L plants showed that callus development could be achieved at 83%-100% with media treatment with the addition of 3 mg BAP and 0.5 NAA, but the explants used were hypocotyl and cotyledon [8]**.**

For culture media treated with 2.4 D (1ml/L and 2 ml/L) callus formation can be seen from irregular shape changes in the explants and they continue to regenerate in size, whereas for the combination media BAP (2ml/L.4ml/L) and 2,4 D (1ml/L, 2ml/L) until the observation period of 4 weeks there was still swelling in the explants and did not show changes in callus formation except in culture media with a combination of BAP2+2,4D2 and BAP4+ 2,4D2 showing swelling followed by formation callus with a small percentage when compared to culture media treated with 2.4D only.

The addition of low concentrations of auxin to the segments/nodes will stimulate callus formation. On the other hand, if the ratio of auxin and cytokinin in the medium is higher, it will stimulate callus explants to regenerate to form organs other than callus, namely shoots [9]. The growth of callus and shoots can be seen in Figure 1.

The leaf stalk explants cut from dioscorea sweet potato plantlets showed success in MS with the addition of Kinetin [7]. Judging from the results of the research that has been carried out, it can be concluded that the concentration of 2.4 D which is lower than the concentration of BAP cannot balance the function of each of these growth regulators.

For the leaf explants themselves, callus formation did not occur on all experimental media, indicated by the absence of a response to swelling, even the edges of the leaves began to turn black, indicating that the explant tissue was starting to die. Each plant cell contains genetic information and certain physiological facilities that are capable of forming a complete plant when placed in a suitable environment. The research from various types of explant sources (young leaves, stem segments, root segments, leaf petioles and axillary buds or nodes) obtained callus growth from axillary shoot material (nodes). Meanwhile, the leaves also did not experience callus growth [10]. The condition of leaf explants in various cutting media can be seen in Figure 2.

Explants from the leaves will not grow callus because the media conditions do not support callus growth. This can occur due to incompatibility of explants with nutrients, vitamins, or inappropriate concentrations of growth regulators (too high or too low). This shows that the need for growth regulators required for callus induction varies depending on the type of explant and type of plant. So, it is necessary to optimize the type and concentration of ZPT to obtain maximum callus growth. The research explained [11], that the best value obtained for the growth of *cimplukan* leaf callus was a combination of 2,4-D and BAP concentrations, namely giving a 2,4-D concentration of 1 mL/L with the addition of 2 mL of BAP. /L concentration. However, for *gembili* leaves in research that has been carried out, the concentration or combination of PGR 2.4 D and BAP has not been able to stimulate callus growth from leaf explants.

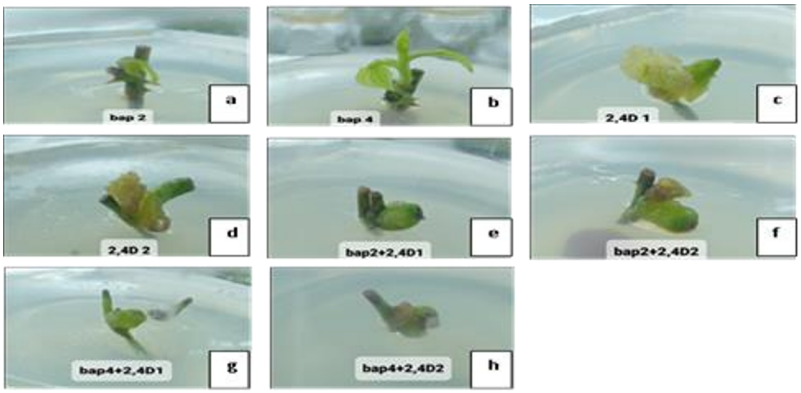

Fig. 1. Callus and shoot growth on various media with nodal explants. (a) BAP 2mL/L (b) BAP 4mL/L (c) 2.4D 1mL/L (d) 2.4D 2mL/L (e) BAP 2mL/L+2.4D 1mL/L (f) BAP 2mL/L +2.4D 2mL/L (g) BAP 4mL/L+2.4D 1mL/L (hour) BAP 4mL/L+2.4D 2mL/L.

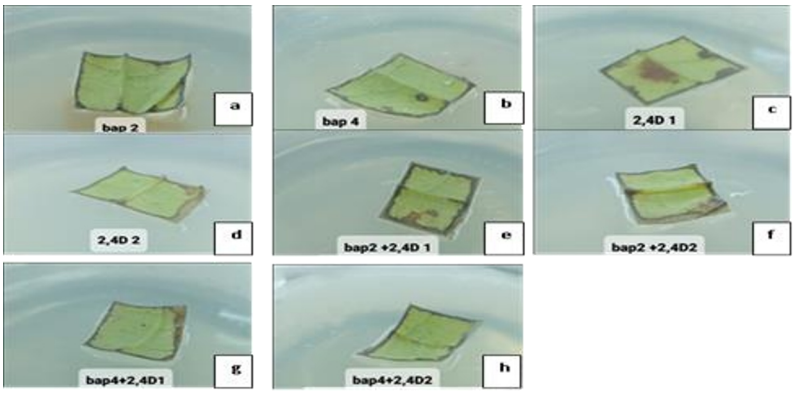

Fig. 2. Appearance of leaf explants in various treatment media (a) BAP 2mL/L (b) BAP 4mL/L (c) 2.4D 1mL/L (d) 2.4D 2mL/L (e) BAP 2mL/L+ 2.4D 1mL /L (f) BAP 2mL/L+2.4D 2mL/L (g) BAP 4mL/L+2.4D

Observation of the results of callus growth on internode and leaf explants can be seen in Figure 3/

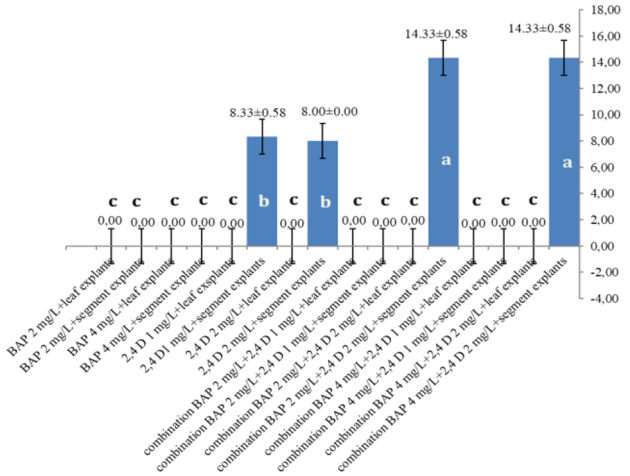

Fig. 3. Histogram of callus growth results on various culture media compositions
Note: Numbers followed by the same letter are not significantly different in the 5% BNT test.

In the table above, observation results were obtained with a score of 0.00, which means that no callus growth occurred in the treatment media. In media treated with BAP without the addition of 2.4 D for the source of explant segments, growth regeneration into shoot growth occurred so that the data was set at zero. The same results were also obtained for leaf explants in all treatment media, there was no change in the direction of callus growth so a score of zero was written.

The observation results showed that the best treatment was the explant segment of culture media with 2.4D 2 mL.L^{-1} treatment with a score of 8.00 but it was not significantly different from the 2.4D mL.L^{-1} treatment media with a score of 8.33. followed by BAP+2.4D combination culture media treatment, namely BAP 2 mL.L^{-1} + 2.4D 2 mL.L^{-1} mL.L^{-1} and BAP 4 mL.L^{-1} and 2.4D 2 mL.L^{-1} with an average score of 14.3. for the combination of BAP 2 mL.L^{-1} + 2.4D 1 mL.L^{-1} and BAP 4 mL.L^{-1} + 2.4D 1 mL.L^{-1}, there was no callus formation, only swelling. This shows that the lower concentration of 2.4 D has not been able to compensate for the higher concentration of BAP in callus formation.

3.2 Quantitative Observation of Callus Diameter

Observation of the callus diameter is carried out to see the development of the callus. A callus that is developing indicates that the cells formed are still alive. The addition of exogenous PGR is intended to stimulate faster callus development. The interaction of auxin and cytokinin with endogenous hormones determines explant growth patterns. The amount and type of PGR provided will determine which endogenous PGR in the explant will support or hinder PGR performance. The interaction of exogenous ZPT provided with endogenous hormones by plant cells will determine the direction of culture developmentl [3].

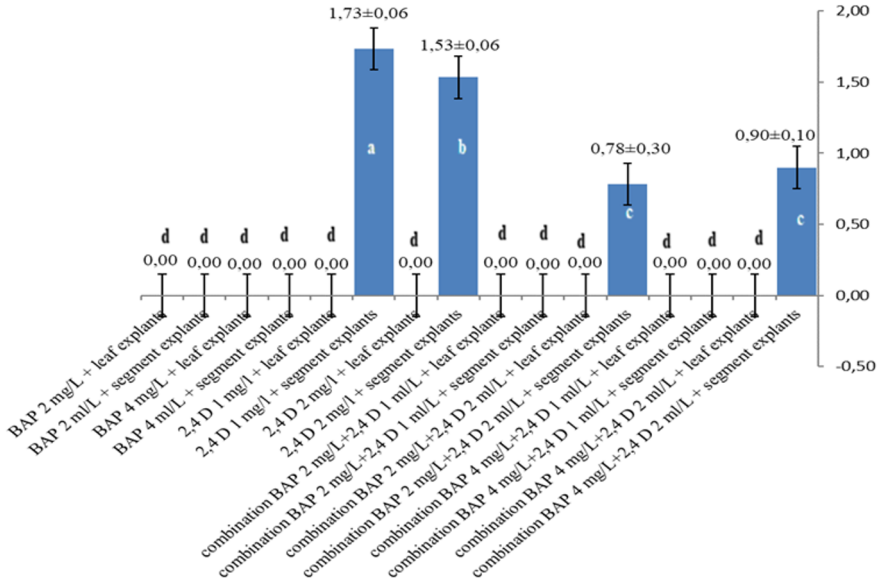

Fig. 4. Histogram of the results of measuring the callus diameter of West Papua gembili plants on various culture media compositions.
Note: Numbers followed by the same letter are not significantly different in the 5% BNT test.

The process of callus formation cannot be separated from cell division, enlargement and elongation. Auxin plays a role in callus formation, so that auxin can increase the permeability of cell walls, so that water, organic ions and inorganic molecules can enter the cells. This is characterized by an irreversible increase in the size and dry weight of the callus. Growth is

related to an increase in the volume and number of cells, the formation of new protoplasm, an increase in body weight, and a subsequent increase in dry weight [12].

From the results of research that has been carried out on media treated with 2.4 D 1 mL.L^{-1}, it shows that the best results are the media treated with 2.4D 2 mL.L^{-1} mL.L^{-1} and finally the media with the combination of BAP + 2.4D. Observation of callus diameter on internode and leaf explants can be seen in Figure 4.

From the table above it can be concluded that the addition of BAP to media containing 2,4D inhibits callus growth because growth regulators from the cytokinin group at low doses cause shoot growth. The addition of BAP 2 mL.L^{-1} and 4 mL.L^{-1} cannot help callus formation but inhibits callus growth. Because the functions of cytokinin and auxin ZPT are opposite to each other, the addition of BAP concentrations of 2 mL.L^{-1} and 4 mL.L^{-1} is a low concentration, low concentrations of cytokinin will affect shoot growth but the use of high concentrations of PGR will stimulate explants to grow. grow. causes shoot growth [13].

Carried out tests on Ananas comosus leaves with the addition of the growth regulator 2,4-D and kinetin, the results showed that administration of 2,4-D without kinetin produced the highest callus height [14]. This shows that administration of 2,4 -D has an effect on callus height. The addition of 2,4-D to the media can stimulate cell division and enlargement, thereby encouraging callus formation and growth. The addition of the growth regulator substance auxin 2.4 D to the culture media can accelerate the process of cell division and cell enlargement in cultured plant explants so that callus formation and growth increases (1).

3.3 Quantitative Observation of Callus Weight

Physiological fresh weight consists of two ingredients, namely water and carbohydrates. The large fresh weight of callus is caused by the high water content. The fresh weight produced is very dependent on the speed of the cells dividing, multiplying and continuing to enlarge. Calluses [15].

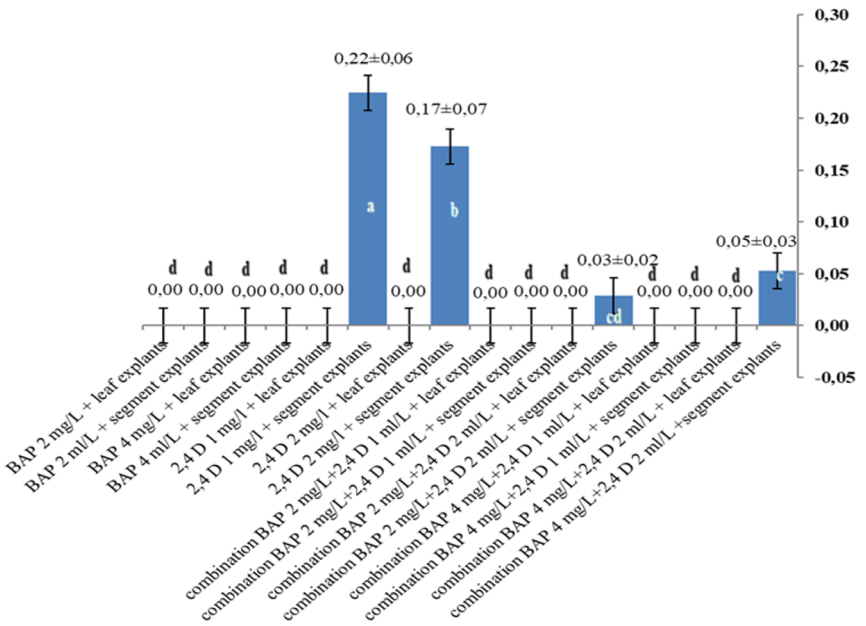

Fig. 5. Histogram of observations of callus weight of West Papuan gembili plants on various culture media compositions
Note: Numbers followed by the same letter are not significantly different in the 5% BNT test.

The optimal cell division can produce optimal callus growth thereby increasing the fresh weight of the callus [16]. The results of different callus weights show that cells in plants have different responses to their ability to absorb water. Observation of callus weight on internode and leaf explants can be seen in Figure 5.

4 Conclusion

The administration of 2.4 D greatly influenced the callus formation of gembili plants, and West Papua accessions from internode explants, however the concentration of 2.4 D did not fully affect callus growth from leaf explants. The application of BAP had no effect on the callus formation of West Papuan gembili plant accessions from internode and leaf explants, however BAP concentration showed regenerative growth of axillary shoots from internode explants. The results of the research that has been carried out show that the composition of the media with the addition of 2.4 D 2 $mL.L^{-1}$ produces an average of 8.00 days of callus growth, however, for callus growth the culture media with the addition of 2.4 D $mL.L^{-1}$ produces a value best. 1.7333 cm for callus diameter and a value of 0.2247 gr for callus weight.

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