# Evaluation of *in vitro* Growth and Development of Banana cv. Barangan Plantlets as Affected by Biotin Application

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**Abstract.** The presence of vitamins in culture medium has proven to be important during tissue culture of variuous species, including banana. Although there are many literatures on banana tissue culture, specific studies on banana cv. Barangan are limited. Through this study we investigate the effect of Biotin as a source of vitamins on plantlet regeneration through tissue culture. The study was conducted at the Plant Biotechnology Laboratory, University of Jambi. Plant material used were slices of banana cv. Barangan sucker cultured on MS basal medium. Biotin was tested at 0.0, 0.5 and 2.5 mg·L$^{-1}$. The trial was arranged in a completely randomized design with 10 replications, in which each experimental unit consisted of 4 cultures. Variables observed were callus proliferation (proliferating time, color, structure, and texture), shoot formation (time of emergence of shoots, number of shoots and length of shoots), and root formation (time of emergence of roots, number of roots and length of the longest roots). Data collected during the study were analyzed using Analysis of Variance followed by the Least Significant Difference test at 5% confidence interval to see the difference in effect between treatment means. The results of the study showed that, the rate of callus proliferation on the explants of banana cv. Barangan could be enhanced by the addition of Biotin to the culture medium. In addition, Biotin application up to 2.5 mL$^{-1}$ was proven to be effective to promote the growth and development of banana cv. Barangan plantlets in tissue culture system.

**Keywords:** Plant Micropropagation, Plantain, *Musa* sp., Growth Substances, Vitamin.

## 1 Introduction

Banana is one of horticultural plants that have a good prospect to be developed in Indonesia. It is a tropical fruit crop that is in great demand, both for domestic and overseas markets. Bananas can be served as "table fruit" or in various forms of processed products. Besides its sweet and fresh taste, the nutrients contained in bananas are high and complete, such as carbohydrates, protein, fat, cellulose, starch and tannins, vitamin A,


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vitamins B1, B2, B6 and B12, vitamin D, vitamin C, calcium, phosphorus, iron, sodium, potassium, magnesium, and zinc [1].

Efforts to increase banana fruit production require large amounts of seeds. The seeds must meet the requirements in terms of health (disease-free) and have uniform growth. Provision of seeds through generative propagation in banana plants is not possible because this plant does not produce seeds (parthenocarpy) except under certain conditions. Whereas conventional vegetative propagation using suckers is faced with the limited availability of parent material.

An alternative technology that can be applied in the propagation of banana seedlings is tissue culture technique. The advantage of this technique is that it can produce plants massively, uniformly and in a relatively short time [2-4]. In addition, the progeny produced are also free from pests and diseases, especially those caused by bacteria and fungi [5,6]. In the propagation banana plants, the tissue culture technique has been implemented with varying degrees of success.

One of handicaps in tissue culture method for plant propagation is that the technology is determined by the involvement of growth regulators and vitamins for its success. The presence of the vitamin in culture media has proven to be important to note tissue culture. Abdelsalam *et al.* [7] suggested that vitamins are important in the regulation of plant growth and development. Vitamins act as catalysts that accelerate metabolism and increase cell differentiation. In tissue culture system, plants produce vitamins in suboptimum amounts, so vitamins need to be added to culture medium to meet the needs of explant cells [8].

Biotin is one of the vitamins that is often used in the application of tissue culture techniques for plant propagation [9]. Biotin, also known as vitamin H or vitamin B7, is an important vitamin that acts as a cofactor for carboxylase, decarboxylase, and transcarboxylase enzymes in plant metabolism [10]. The use of Biotin in tissue culture was reported by Yelnititis [11] where the application of 2,4-D + Biotin resulted in very crumbly and yellowish callus on ramin (*Gonystylus bancanus*) explants. The use of Biotin has also been reported in study by Samarina *et al.* [12] who found that the addition of Biotin 1 – 3 mg $L^{-1}$ could increase shoot length in *Gerbera jamesonii* tissue culture. Further, Al-Khayri [8] used vitamins Biotin and Thiamine to test the effect of their combination on callus growth and somatic embryogenesis of dates (*Phoenix dactylifera*) using MS media. The results showed that embryogenic callus weight, number of embryos produced, and embryo length were affected. significantly by the concentration of Thiamine and Biotin. The optimum treatment for callus growth was obtained at a concentration of 0.5 mg $L^{-1}$ Thiamine plus 2.0 mg $L^{-1}$ Biotin. This treatment also gave the highest number of embryos. Embryo elongation was greatest at 0.5 or 2 mg $L^{-1}$ Thiamine in combination with 1.0 mg $L^{-1}$ Biotin.

The use of Biotin has also been reported successful at the Plant Tissue Culture Laboratory of the Dinas Tanaman Pangan dan Hortikultura (DTPH), Tanjung Jabung Barat Regency, Jambi Province, and has become part of the Technical Guidelines for Banana Propagation through Tissue Culture Techniques. DTPH Tanjung Jabung Barat is one of agencies that regularly propagates bananas through tissue culture method. They recommended that BAP 1.0 mg·$L^{-1}$ was used as the basic treatment in combination with Biotin at 0.5 to 2.5 mg·$L^{-1}$.

Although there are lots of literatures on banana tissue culture, specific studies on banana genotypes from Barangan cultivars are still limited. In fact, this banana cultivar has high economic value because of its sweet fruit taste and long shelf life, so it is very popular among banana consumers. Therefore, through this research we would like to study the effect of Biotin and find out the best Biotin concentration to support the growth of sucker explants in banana cv. Barangan tissue culture.

## 2 Materials and Methods

The materials used in this study were banana shoots (*Musa acuminata* L) cv. Barangan. The medium used was the Murashige and Skoog (MS) [13] basal medium solidified with Bacto Agar and provided with 30 g $L^{-1}$ sucrose. The disinfectants used were sterile water, 70% and 95% alcohol, and NaOCl. The vitamin to be tested was Biotin, while 1 mg·$L^{-1}$ BAP was used as basic treatment.

Laboratory equipment used in this study were culture flasks, Erlenmeyer glass, Beaker glass, volumetric flask, pipette, analytical balance, pH meter, autoclave, magnifying glass, oven, laminar air flow cabinet (LAFC), hot plate with magnetic stirrer, spirit lamps, and dissecting sets (tweezers, scalpel handles and scalpel blades).

### 2.1 Experimental Design

This trial used a single-factor Completely Randomized Design (CRD). Biotin as the treatment tested at various concentrations: 0.5 mg·$L^{-1}$, 1.0 mg·$L^{-1}$, 1.5 mg·$L^{-1}$, 2.0 mg·$L^{-1}$ and 2.5 mg·$L^{-1}$ and a control (without Biotin). Each treatment was repeated 4 times, so there were 24 experimental units. Each unit consisted of 4 cultures, making the total of 96 cultures.

### 2.2 Culture Initiation

Explants were cultured in a sterile manner in the LAFC. Prior to culture on media supplemented with different concentration of Biotin, all explants were first cultured on MS 0 medium (precondition medium) for 1 month. This was aimed to see culture development, especially in relation to the occurrence of browning and microorganisms contamination.

Sterilized explants with a length of 2.5 – 3.0 cm and a diameter of 2.0 cm were split into two equal parts (same size). The explants were dissected in a 1% vitamin C solution, then dried up on sterile filter paper before being inoculated on prepared medium. The culture flasks were capped with aluminium foil and tied up with a rubber band, labelled according to the treatment and date of planting. The cultures were then maintained in a culture room with a room temperature of 25 ± 1 ºC and a photoperiod of 16 hours per day.

### 2.3 Observation

Observations were made every day to see whether there was contamination, and to identify the initial appearance of browning, callus proliferation, formation of shoots, roots, and others. Data, both quantitative and qualitative were recorded according to scheduled time. In addition, data were also captured in visually in the form of picture of explant development during study.

The variables observed in this study were: 1) callus formation which included time from culture initiation to first callus proliferation, callus colour, callus texture and callus structure, 2) shoot formation and development which included time of shoot first emergence from culture initiation, number of shoots formed and shoot length, and 3) root formation and development which includes the time of root emergence from culture initiation, number of roots formed and the length of the longest root.

### 2.4 Data Analysis

Analysis of Variance (ANOVA) was used for all variables by using the Microsoft Excel Spreadsheet application [14]. Furthermore, to see the difference of the effect between treatments, a Least Significant Difference (LSD) test was carried out at $\alpha = 0.05$ [15].

## 3 Results and Discussion

### 3.1 Media Browning

Results showed that all explants turned brown or blackish-brown following 4 weeks of culture initiation (figure 1). Browning in plant tissue can be caused by enzymatic [16] or non-enzymatic [17] reaction, depending on the involvement of enzymes in the process. However, enzymatic browning seemed to be the main cause of explant browning in most tissue culture system [18].

Browning is common phenomena in tissue culture of banana due to the release of phenolic compounds by the explants which get oxidized [19-21]. The release of phenolic compounds is a natural response to wounding the plant materials in order to remove explants. This response could be toxic to plant tissues, reduced cell division and explant growth [22], and resulting in explant death eventually [23]**.**

When explant is isolated from stock plant materials, it causes wounding of tissues. This wounding further facilitates the release of various enzymes such as polyphenol oxidase, superoxide dismutase, and peroxidase as a mechanism of defence [24]. These enzymes come to the rescue of the plant by catalysing various reactions to eliminate reactive oxygen and heal the plant part. One of these reactions results in the production of melanin [25]**,** a dark pigment, which results in the browning of explants. These exudates form a barrier around the cultured tissue that inhibit nutrient uptake [26,27]. Further, they leach into the medium and result in the browning of the media.

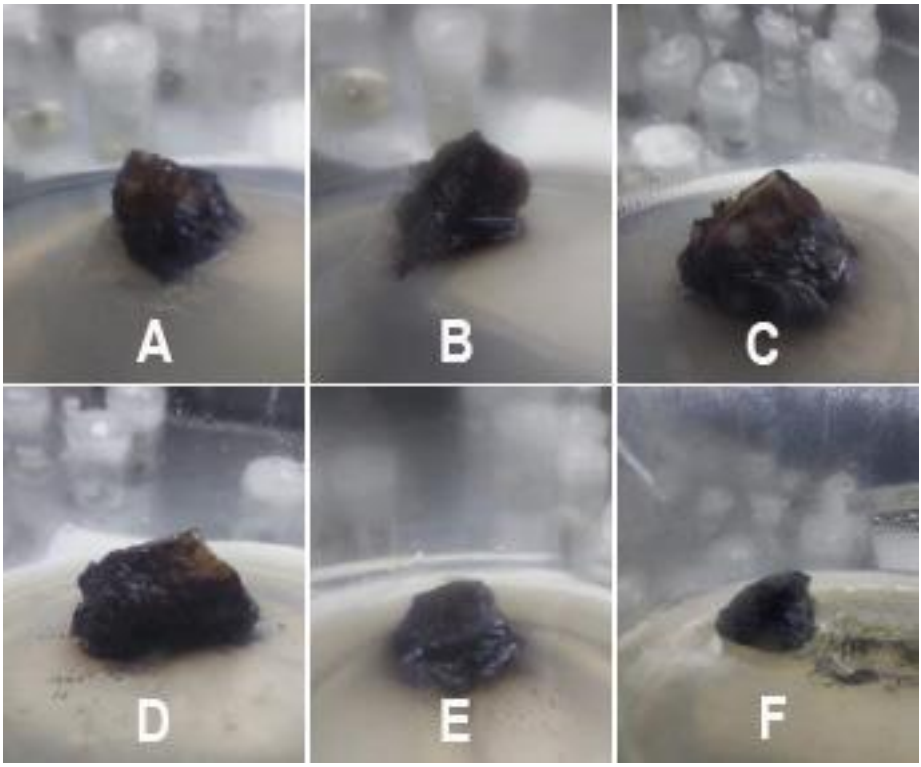


**Fig. 1.** Browning that occurs in banana explants due to the release of phenolic compounds from the cultured tissue (A=0.5 mg·L$^{-1}$, B=0.5 mg·L$^{-1}$, C=1.0 mg·L$^{-1}$, D=1.5 mg·L$^{-1}$, E=2.0 mg·L$^{-1}$, F=2.5 mg·L$^{-1}$).

Browning is one of the serious problems in plant propagation through *in vitro* culture that frequently led to unsuccessful plant propagation. In order to control the browning some efforts could be done such as pre-soaking explants in antioxidant solution, application of antioxidants in culture medium, maintaining explants in the dark condition for a period of time, and subculturing explants in new fresh medium. Pre-soaking of explants in antioxidants such as polyvinylpyrolidone and ascorbic acid is found to solve the problem of browning [28]. The application of antioxidant and activated charcoal in culture medium is often used to control browning in various plant. In addition, frequent sub culturing and keeping explants in total dark condition for certain period is also suggested to control explant browning. In current study we used ascorbic acid solution to pre-soak explant materials during preparation.

### 3.2 Callus Proliferation

Callus proliferation is important step in inducing somatic embryogenesis in plant propagation by tissue culture. The production of embryos through somatic embryogenesis could only succeed on callus with embryogenic properties. The application of growth

substances to modify culture medium could resulted in embryogenic callus formation leading to the production of somatic embryos [2]. Thus, enrichment of culture medium with growth substances such as Biotin is beneficial to improve embryogenic callus proliferation in banana tissue culture.

It was found that the level of Biotin incorporated in culture medium was crucial for callus proliferation on banana sucker explants. In all levels of Biotin callus first proliferated at the wounded edges after tissue swelling. The formation of callus on the surface of wounded tissues is presumably due to the action of tissue recovery stimulus. This is in accordance with Zulkarnain's [4] suggest that callus developed in tissue culture system was due to hormonal response on tissue injury. The overall callus induction ranged from 16.67 to 75.00 % (Table 1). The application of 1.5 mg.$L^{-1}$ Biotin resulted in the highest percentage of explants forming callus (75.00 %), while fastest callus proliferation was found on control that was approximately 16 days from culture initiation (Table 2). Proliferated callus in all treatments showed relatively similar characteristics. They are white in color and dominated by compact structure (see Fig. 2)

**Table 1.** The effect of different levels of Biotin on the percentage explant forming callus in tissue culture of banana cv. Barangan.

| Biotin concentration | Number of explant forming callus (%) |
|---|---|
| 1.0 mg.$L^{-1}$ | 75.00 ± 8.33 a |
| 0.0 mg.$L^{-1}$ | 58.33 ±19.25 a |
| 0.5 mg.$L^{-1}$ | 58.33 ±11.11 a |
| 2.5 mg.$L^{-1}$ | 35.83 ±11.66 ab |
| 1.5 mg.$L^{-1}$ | 16.67 ± 9.62 bc |
| 2.0 mg.$L^{-1}$ | 16.67 ± 9.62 bc |

$LSD_{0.05}$ = 35.92, ± Standard Error

**Table 2.** The effect of different levels of Biotin on the time of callus proliferation on sucker explants of banana cv. Barangan.

| Biotin concentration | Time of callus formation (days after subculture) |
|---|---|
| 0.0 mg.$L^{-1}$ | 15.96 ± 1.35 a |
| 0.5 mg.$L^{-1}$ | 17.88 ± 1.64 ab |
| 2.5 mg.$L^{-1}$ | 18.04 ± 1.85 ab |
| 2.0 mg.$L^{-1}$ | 18.50 ± 0.50 ab |
| 1.0 mg.$L^{-1}$ | 18.83 ± 1.09 ab |
| 1.5 mg.$L^{-1}$ | 22.00 ± 2.00 b |

$LSD_{0.05}$ = 35.92, ± Standard Error

Al-Khayri [8] reported the use of vitamins Biotin and Thiamine to test the effect of their combination on callus growth and somatic embryogenesis of dates (*Phoenix dactylifera*) on MS medium. The results showed that callus weight, number of embryos, and embryo length were significantly affected by the concentrations of these two vitamins. The optimum treatment to support callus growth was obtained at 0.5 mg·$L^{-1}$ Thiamine + 2.0 mg·$L^{-1}$ Biotin. This treatment also produced the highest number of embryos, while the greatest embryo elongation was obtained with 0.5 or 2 mg·$L^{-1}$ Thiamine in combination with 1.0 mg·$L^{-1}$ Biotin. This is in accordance with our finding

where the application of up to 1.5 mg·L$^{-1}$ Biotin produced the highest number of explant forming callus (75.00 %) but longer time required for callus proliferation (up to 22 days after subculture).

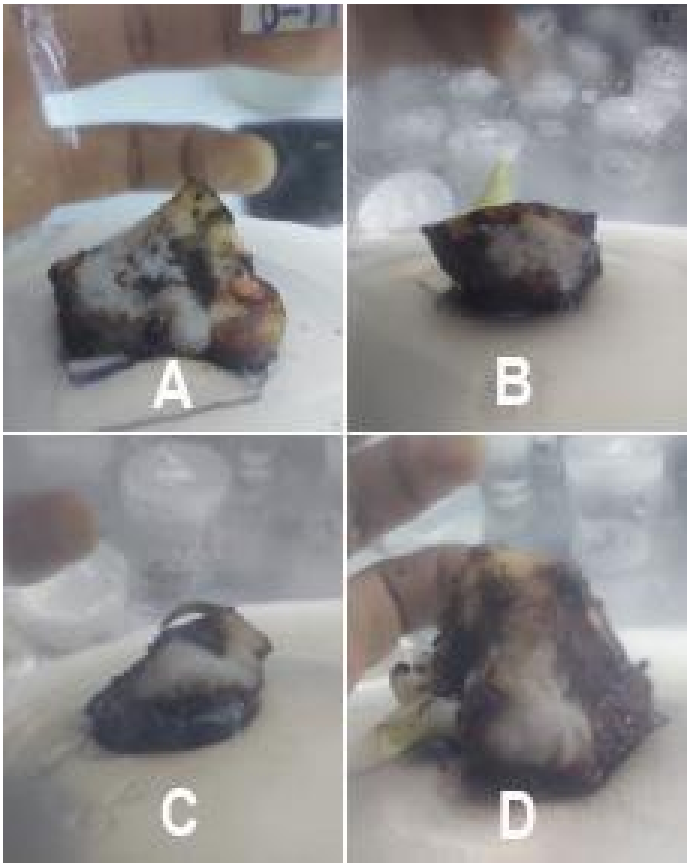


**Fig. 2.** Browning that occurs in banana explants due to the release of phenolic compounds from the cultured tissue (A=0.5 mg·L$^{-1}$, B=0.5 mg·L$^{-1}$, C=1.0 mg·L$^{-1}$, D=1.5 mg·L$^{-1}$, E=2.0 mg·L$^{-1}$, F=2.5 mg·L$^{-1}$).

### 3.3 Shoot Formation

Analysis variance showed that there was no significant effect of Biotin on the percentage of explant forming shoot (P-value = 0.18). The effect of Biotin application on the percentage of explant forming shoot is presented on Table 3.

**Table 3.** The effect of different levels of Biotin on the percentage of sucker explants of banana cv. Barangan forming shoots.

| Biotin concentration | Number of explant growing shoots (%) |
|---|---|
| 1.0 mg.L$^{-1}$ | 77.78 ± 25.00 |
| 1.5 mg.L$^{-1}$ | 66.67 ± 13.61 |
| 2.0 mg.L$^{-1}$ | 66.67 ± 21.52 |
| 2.5 mg.L$^{-1}$ | 50.00 ± 15.96 |
| 0.0 mg.L$^{-1}$ | 33.33 ± 9.62 |
| 0.5 mg.L$^{-1}$ | 22.00 ± 9.62 |

$LSD_{0.05}$ = 35.92, ± Standard Error

On the other hand, the time of shoot regeneration on the explants was significantly affected the presence of Biotin in culture media (*P*-value = 0.02). The fastest explants forming shoots were those culture on media supplemented with 0.5 mg.L$^{-1}$ Biotin (17 days after subculture on the average). The longest time to regenerate shoots was found on explants cultured on media with 2.5 mg.L$^{-1}$ Biotin (Table 4).

**Table 4.** The effect of different levels of Biotin on the time of shoot formation on sucker explants of banana cv. Barangan.

| Biotin concentration | Time of shoot formation (day after subculture) |
|---|---|
| 0.5 mg.L$^{-1}$ | 17.00 ± 2.00 a |
| 0.0 mg.L$^{-1}$ | 21.00 ± 6.00 ab |
| 1.5 mg.L$^{-1}$ | 21.96 ± 0.91 ab |
| 2.0 mg.L$^{-1}$ | 22.22 ± 0.78 ab |
| 1.0 mg.L$^{-1}$ | 24.89 ± 1.25 b |
| 2.5 mg.L$^{-1}$ | 32.75 ± 2.75 c |

$LSD_{0.05}$ = 35.92, ± Standard Error

Explants browning seemed not to inhibit shoot formation. Though there was browning, the explants still show the signs of life tissues and were able to regenerate shoots (see Fig. 3). The shoots looked healthy with green or whitish green colour and appeared from brown or dark brown explants.

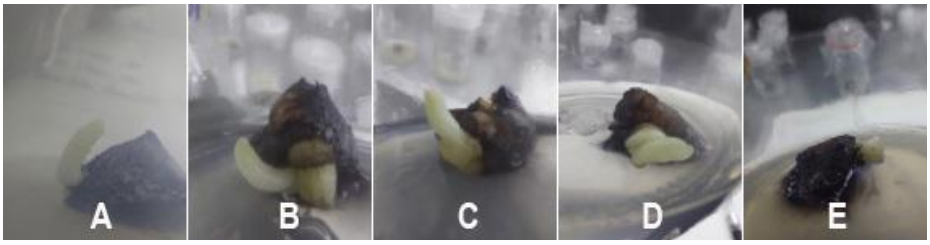


**Fig. 3.** Shoots formation on sucker plantlets of banana cv. Barangan after 8 weeks of culture (A=0.5 mg·L$^{-1}$, B=1.0 mg·L$^{-1}$, C=1.5 mg·L$^{-1}$, D=2.0 mg·L$^{-1}$, E=2.5 mg·L$^{-1}$).

The use of Biotin in plant tissue culture media has been studied by several researchers [7, 8, 12, 29]. Samarina *et al.* [12] reported that Biotin application at 1 - 3 mg·L$^{-1}$ in MS

medium increased the length of *Gerbera jamesonii* shoots up to 6 cm. Meanwhile Abdelsalam *et al.* [7] proved that the addition of Biotin to MS media could increase the number and length of *Cymbopogon schoenanthus* roots. Thepsithar *et al.* [19] also reported the use of 0.5 $mg \cdot L^{-1}$ Biotin was able to encourage the growth of protocorm-like bodies in the *Phaleonopsis* Silky Moon orchid. Therefore, it is clear that media enrichment with vitamin Biotin is beneficial to support *in vitro* culture of agricultural crops.

## 4 Conclusion

It can be concluded that the enrichment of culture media with vitamin Biotin is beneficial for growth and development of sucker explants of banana cv. Barangan in tissue culture system. In addition, the application of 1.0 - 2.0 $mg.L^{-1}$ of Biotin could give the best response in term of shoot formation on sucker explants of banana cv. Barangan.

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