


Research Article

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# Direct Organogenesis in Local Clones of Patchouli Plant (*Pogostemon cablin* Benth) In Vitro

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## Abstract

Growth regulators that are often used in tissue culture to increase the production of secondary metabolites (organogenesis), such as shoot multiplication are cytokinin. Cytokinin growth regulators include Benzyl Amino Purine (BAP) and kinetin. The objective of this research is to obtain the best concentration of BAP interaction with Kinetin concentration, the best concentration of BAP, and the best concentration of Kinetin that can stimulate the formation for multiplication of patchouli plants. The concentration of BAP in the in vitro growing medium varies according to the type of plant and the type of explants used. The cytokinin concentrations used ranged from 0.1-10 mg/L media. The research material used in this study was the accession of patchouli plants in West Sumatera, namely Situak and method of research is a completely randomized design. The treatment will be tested in this study consist of BAP concentrations with 5 levels (0.5, 1, 1.5, 2, 0 mg/L, and concentration of Kinetin with 2 levels (0.5, 1 mg/L). The basic media used were Murashige and Skoog (MS). Concentrations of 0.5 mg/L kinetin + 0.5 mg/L BAP, 0.5 mg/L kinetin + 1 mg/L BAP and 1 mg/L kinetin + 1 mg/L BAP can stimulate organogenesis (bud) in vitro. The results showed that the concentration of Kinetin and BAP influenced the growth and development of explants.

Keywords: BAP, growth regulators, kinetin, patchouli plant, tissue culture



## 1. Introduction

Patchouli plants (*Pogostemon cablin* Benth) is the perennial herbs (Wahyudi and Ermia, 2012). It's the main raw in the pharmaceutical industry and the essential oils of the word (Huang, et al., 2016). Living in the subtropics, growing well at altitudes of up to 1,200 m asl in warm and wet climates with rainfall between 1,500 mm to 3,000 mm annually evenly throughout the year, 70-90% humidity, 24-28°C temperatures. Good soil types are regosol, latosol, and alluvial. Sandy clay texture or clay dusty, have good absorption and is not inundated during the rainy season with soil pH 5.5-7.0. It grows wild in several parts of the world. It grows wild in Malaysia, Indonesia, and Singapore as well (Ramya, et al. 2013).

In Indonesia, patchouli plants spread of Aceh and West Sumatera, especially in West Pasaman. There are seven locations in West Pasaman Regency where the community has known for a long time and are still familiar with and still have a high interest in patchouli cultivation and refining business at the foot of Mount Pasaman (Hidayat, Mayerni and Syarif, 2017). West Sumatra. According to Febriyetti, Mayerni, and Anwar (2017), from the observations of plant quality characteristics, it can be concluded that Rimbo Binuang accession and Situak can be used for the best clone since the highest oil yield is obtained in Rimbo Binuang accession and the highest alcohol content of patchouli is found in Situak accession.

Since *Pogostemon cablin* Benth does not flower, the plants are reproduced by cutting. Cutting with 15-20 cm length is inserted into well-prepared soil and shaded until established. The plants are easily rooted without using rooting hormones. However, the horticultural practice of cutting is a slow process and not suitable for large-scale propagation. Therefore, a more rapid method of propagation is required for large scale propagation. One such method of propagation that can be usefully employed to produce relatively uniform plantlets in a short time is via in vitro culture (Fitriana, 2011).

The cultivation of patchouli presents some problems that affect the production of biomass and the yield of essential oil. The low yield of essential oil is due to the plant's susceptibility to different types of viruses and bacterial. Thus, in vitro propagation is a viable alternative for obtaining pathogen-free plants and also allows for large-scale propagation in a relatively short period, through the in vitro propagation (Arrigoni, et al., 2011). And then, In vitro micropropagation is an effective means for the rapid multiplication of plant species of clonal origin (Saha, et al. 2020). This multiplication is determined by many factors including the type of explants and growth regulators (Swammy, 2016, Hua et al. 2014; Norrizah et al. 2012). Many in vitro studies have been reported on different patchouli species, using nodal segment explants. One such method of propagation that can be usefully employed to produce relatively uniform plantlets in a short time is via in vitro culture (Inampudi, et al. 2017). And using growth regulators

that are often used in tissue culture to increase the production of secondary metabolites (organogenesis), such as shoot multiplication are cytokinin. Cytokinin growth regulators include Benzyl Amino Purine (BAP) and kinetin. BAP is a more economical and frequent cytokinin used to stimulate the multiplication of axillary shoots (Pradana, 2011), and an increase in the number of shoots (Tiliar and Sompotan, 2007). And functions of Kinetin to regulate cell division and morphogenesis (Sintha, 2017).

## 2. Materials and Methods

### A. Plant Material and Time of Research

The research was conducted in the Laboratory of Tissue Culture, Faculty of Agriculture, Andalas University from May until November 2019.

Plant material is local *Pogostemon cablin* namely Situak. Media is Murashige and Skoog (MS) and Growth regulators Benzyl amino purine (BAP), and kinetin (KIN). Explants using nodal segment 1-2 cm explants taken from mother plants as that long was suitable for sterilization procedures. Mother plants of Patchouli (*Pogostemon cablin* Benth) age 6 months.

### B. Experimental Details

The research method used a randomized factorial design. In each treatment consist of three replications. The treatment will be tested in this study : 1) BAP, 5 levels concentrations (0.5, 1, 1.5, 2, 0 mg/L) and 2) Kinetin, 2 levels concentrations (0.5 and 1 mg/L). The basic media used were the Murashige and Skoog (MS) base media which were added with 30 g / L sucrose sugar and 8 g / L agar. Sterilization explants, the leaves were removed from the explants and washed under running tap water for 2-3 times to wash off the external dust/contaminants. After sterilization of explants, explants were inoculated in culture bottles aseptically.

Each bottle of culture was filled with 5 explants. The media is autoclaved at a pressure of 20 psi for 15-25 minutes. The culture bottles that have been planted are stored in the culture chamber with a constant room temperature of 26° C. Observe the growth of plant shoots. Observations are carried out every week. The parameters observed were the percentage of life, shoot induction time, and high of bud. Analisis data is only done on treatment response using the F test at 5% level. If the results show a real effect then proceed with the DMRT test (Duncan Multiple Range Test) 5%.

## 3. Results and Discussion

### A. The Percentage of Live Explants (%)

Live explants are characterized by fresh, brightly colored, and not browned explants. The percentage (%) of live explants of patchouli plant accessions for Situak in many concentrations of Kinetin and BAP (Table 1).

**Table 1.** Live percentage (%) of patchouli plant explants in many concentrations

The concentration of Kinetin (mg/l)	BAP concentration (mg /L)				
	0	0.5	1,0	1.5	2.0
	(%)				
0.5	0.0	16.6	16.6	0.0	0.0
1	0.0	0.0	16.6	0.0	0.0

**Table 2.** Table 2. Shoot induction time in many concentrations of BAP and Kinetin

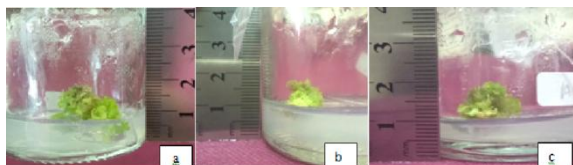
Kinetin concentration	BAP concentration (mg / l)				
	0	0.5	1,0	1.5	2.0
	(HST)				
0.5	-	25	20	-	-
1	-	-	18	-	-

Note: - (Does not bud)

**Table 3.** Shoot induction time in many concentrations of BAP and Kinetin

Kinetin concentration	BAP concentration (mg / l)				
	0	0.5	1,0	1.5	2.0
	(cm)				
0.5	-	0.8	1,0	-	-
1	-	-	1,0	-	-

Note: - (Does not bud)

**Figure 1.** Height of buds in many concentration of kinetin and BAP a) 1 mg/L Kinetin and 1 mg/L BAP b) 0.5 mg/L Kinetin and 0.5 mg/L BAP, and c) 0.5 mg/L Kinetin and 1 mg/L BAP

Based on Table 1., it showed that addition of Kinetin and BAP concentrations that produce live explants with a percentage of 16.6% in the treatment of 0.5 mg/L kinetin + 0.5 mg/L BAP, 0.5 mg/L kinetin + 1 mg/L BAP and 1 mg/L kinetin + 1 mg/L BAP. In this research, BAP and Kinetin concentration can growth live explants but the percentage of growth is not optimal. Its growth is characterized by green explants. The result of Rozalina's research results (2013) related to the percentage of live explants of patchouli plants with NAA and BAP treatment showed that BAP concentration of 1.0 mg / l and BAP 1.5 mg / l were treatments with the highest percentage of live explants reaching 93, respectively. 75%. And the lowest in the treatment of 0.5 mg / l BAP is 81.25%. This explains that explants of life with the highest percentage of life were found in the treatment of BAP concentration of 1.0 mg / l and BAP 1.5 mg / l.

Growth of explants is influenced by many factors such as the environment and growth regulator, especially the balance between cytokinins and auxins both exogenous and endogenous explants in tissue culture. In

this research, some explants did not grow until the end of the study. It is caused by contamination. Contamination of plant material culture occurs because of external or internal infection. According to Lina et al. (2013), effort prevention of external contamination is carried out by sterilizing the surface of the plant material. Internal infection cannot be eliminated with surface sterilization. Explants that contain or are infected with bacteria, viruses, or fungi will cause contamination at the growth stage. Even at times early after inoculation, there is no contamination, the next few days fungi growth seen.

### B. Shoot Induction Time

The observations showed that from the 10 combinations of media used, it was obtained that the shoots appeared on the 18-25 days after culture. There are 3 media containing 0.5 mg/L kinetin and 0.5 mg/L BAP, 0.5 mg/L kinetin and 1 mg/L BAP and 1 mg/L kinetin and 1 mg/L BAP which are able to induce buds. The shoot induction time is relatively slow compared to shoot induction in patchouli plants beginning with the emergence of callus in the second week after culture using media containing NAA 0.1 -0.9 mg/L (Isnaeni et al. 2018). The difference in response is due to the effectiveness of each plant growth regulator used. In agreement with the previous report that the use of picloram with a concentration of 2-8 mg/L can induce patchouli leaf callus at 12 DAP (Musdalifah, 2017). The induction time of patchouli explant buds at various concentrations of kinetin and BAP (Table 2).

The function of cytokinins for organ formation, and bud formation (George et al., 2008). According to Rainiyati et al. (2007), the higher the concentration given cytokinins then the number of shoots are formed will be increasing, however, the formation of individual shoots can be hampered so that the correct concentration is determined very need to pay attention to produce maximum multiplication.

### Height of Buds

Observation results showed that the growth of patchouli buds at the age of 8 MST was 0.8 cm -1 cm on the media 0.5 mg/L kinetin + 0.5 mg/L BAP, 0.5 mg/L kinetin + 1 mg/L BAP and 1 mg/L kinetin + 1 mg/L BAP . This is similar to the study of Sobardini et al. (2005) who reported shoot or bud height at treatments 0 and 0.5 mg/L BAP added 0.0.1 and 1 mg/L NAA which resulted in an average shoot height of 0.5 cm-2.18 cm (Table 3).

The difference in growth response of shoot height or shoot length is influenced by different endogenous cytokinin content and the response plus exogenous cytokinin are also different, the meristematic level of explant tissue used is also likely different. Rai et al. (2009) reported that medium containing 1 mg/L BAP was the most effective for shoot multiplication in guava (*Psidium guajava* L.) Patchouli shoot height growth at several concentrations of kinetin and BAP (Figure 1.)

### 4. Conclusions

The results showed that concentration of Kinetin and BAP influenced the growth and development of explants. Concentrations of 0.5 mg/L kinetin + 0.5 mg/L BAP, 0.5 mg/L kinetin + 1 mg/L BAP and 1 mg/L kinetin + 1 mg/L BAP can stimulate organogenesis (bud) in vitro.

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