



# The Combination Effect of Auxin and Cytokinin on Callus Induction of Patchouli (*Pogostemon Cablin Benth.*) from Leaf Explants

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**Abstract.** Patchouli, also known as *Pogostemon cablin Benth.*, is a plant that yields patchouli oil, a crucial essential oil used in the perfume industry. Stem cuttings are the conventional method of vegetative propagation for patchouli plants. Tissue culture techniques offer alternative approaches for vegetative propagation of better seeds. In vitro approaches for mass multiplication and plant breeding demand a callus culture that has been developed. The purpose of this study was to assess how leaf explants responded to callus formation in vitro caused by a combination of auxin and cytokinins. Leaf explants were cultivated on Murashige and Skoog medium for four weeks with the addition of NAA (0.5, 1, 2, and 3 mg/L) and BA/kinetin (0.5 mg/L). In the second week, the explants began to develop calluses; these calluses were friable, yellowish-green, and yellowish-white. Without NAA, medium was unable to produce callus. Callus development was controlled by the interaction of auxin and cytokinin. The NAA content was correlated with an increase in callus biomass. In comparison to the combination of BA and NAA, the combination of kinetin and NAA produced a larger callus fresh weight and percentage of callus formation. On MS medium enriched with 3 mg/L NAA + 0.5 mg/L kinetin, the maximum callusing was attained.

**Keywords:** BA · callus induction · kinetin · NAA · *Pogostemon cablin Benth*

## 1 Introduction

Patchouli plant is an essential herbaceous widely used in the fragrance industry because of the patchouli oil it produces [1]. Indonesia is currently one of the vital suppliers of patchouli oil in the world. Patchouli production in Indonesia reached 2,100 tons in 2018 and increased sharply in 2019 to 16,800 tons [2]. The projected world patchouli oil demand is estimated to increase by 5% every year [3]. In order to meet market needs, sustainable production system development is needed. One of them is the availability of superior patchouli seeds [4]. Plant tissue culture offers a series of techniques in plant propagation and plant breeding to obtain superior plants [5].

Mass propagation and plant breeding to obtain superior patchouli seeds through in vitro techniques can be done through in vitro mutagenesis or genetic engineering. The availability of established callus culture and plantlet regeneration protocol will assist the application of these two techniques in developing patchouli plants. One of the factors that determine the success of callus induction is the plant growth regulator used. Auxins and cytokinins are growth regulators that are widely used in callus induction [6]. Auxin has a function in stimulating cell division and elongation and is involved in root formation. One widely used auxin is naphthalene acetic acid (NAA), a synthetic growth regulator [7]. Cytokinins are compounds that can increase cell division and differentiation also shoot formation [8]. Benzyladenine (BA) is more stable, more difficult to oxidize by light, cheaper, and easy to obtain. Benzyladenine is also more effective than other cytokinins because it can promote the synthesis of different growth regulators [9]. In comparison, kinetin is a cytokinin involved in cell division and morphogenesis [10].

The growth and development of the culture have influenced the ratio of auxin and cytokinin. The ratios between auxin and cytokinin usually differ by genus and species in plant sensitivity to growth regulators [11]. In general, roots will form when the ratio is high, while shoots are formed when the ratio is low. At the same time, the same concentration of both resulted in the formation of callus [12]. Previous studies obtained the highest callus from patchouli leaf explants on Murashige and Skoog (MS) media given NAA (2 mg/L) and BA (0.5 mg/L), but with a low percentage callus formation, namely 40–50% [13]. Meanwhile, callus growth of *Elephantopus scaber* L. induced from leaf explants and cultured on MS media with NAA (1 mg/L) and kinetin (0.5 mg/L) showed the fastest callus induction time [14]. An essential factor in the growth of callus cultivation is appropriate growth regulators for each plant species. Hence, this study objective is to determine the combined effect of NAA with BA or kinetin on callus formation from patchouli (*Pogostemon cablin* Benth.) leaf explants.

## 2 Materials and Methods

### 2.1 Plant Material

The patchouli plant (*P. cablin* Benth.) of the Lhokseumawe variety used in this study is a collection of the Laboratory of Plant Physiology, Tissue Culture and Microtechnics, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University.

### 2.2 Explants Sterilization and Callus Induction

In this study, the explant used was patchouli leaves. The explant was sterilized with 70% alcohol solution for 1 min followed by bleach (5.25% NaClO) 20% solution for 10 min, then sterile water for 5 min each three times. Sterile leaf explants were cut to a size of 1 cm<sup>2</sup> and cultured on MS media with BA/kinetin (0.5 mg/L) combined with NAA (0.5, 1, 2 and 3 mg/L). Each treatment was repeated 5 times (5 bottles), each bottle was filled with 5 explants. The cultures were incubated at 25 ± 1 °C with a light intensity of 600 lx for 4 weeks. The effect of the combination of auxin and cytokinin was evaluated by observing the first callus formation time, callus morphology, callus percentage formation, and callus fresh weight.

### 2.3 Statistical Analysis

Quantitative data on the callus percentage formation and callus fresh weight were analyzed by ANOVA. If the results showed that there was a significant difference, a further test was carried out, namely Duncan Multiple Range Test (DMRT), at a significance level of 5% ( $P < 0.05$ ).

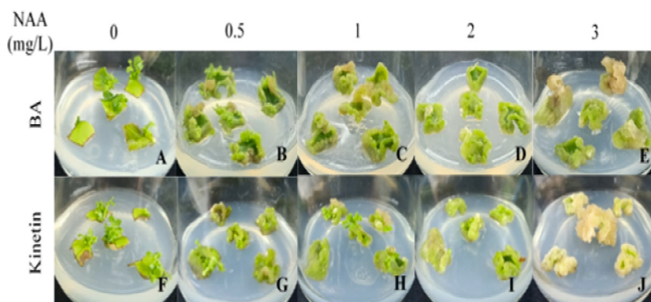
## 3 Results and Discussion

Patchouli callus could be induced from leaf explants cultured on MS media with the addition of growth regulator NAA combined with BA/kinetin (Fig. 1). Callus formation started in the 2nd week after induction. The callus formed was first seen on the cutting edge of the explant, followed by the entire explant surface. The callus formed on the combination medium of BA/kinetin 0.5 mg/L 0.5–2 mg/L was yellowish-green (Fig. 1B–D and G–L), whereas in the combination of BA/kinetin with a high concentration of NAA 3 mg/L is whitish-yellow (Fig. 1J). Media with the addition of BA/kinetin alone without NAA (control) could not induce callus but produced shoots (Fig. 1A and F).

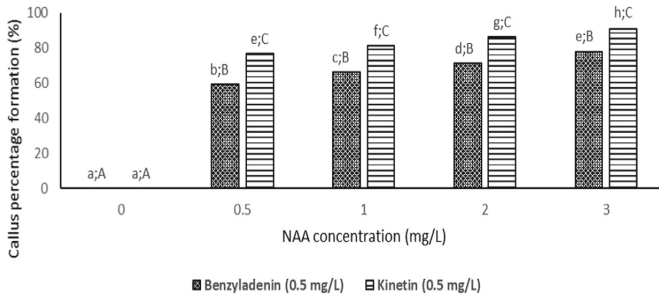
Most leaf explants cultured on MS media combined with NAA and BA/kinetin could form callus. The callus percentage formation from explants cultured on a combination of NAA and kinetin media was higher than that of NAA and BA. The callus percentage formation ranged from 60–80% for MS media with a combination of NAA and BA and ranged from 80–90% for MS media with a combination of NAA and kinetin (Fig. 2). Meanwhile, leaf explants cultured on media with the addition of growth regulators BA/kinetin alone without being combined with NAA could not form callus.

The callus percentage formation was influenced by NAA concentration and the type of cytokinin. The higher the concentration of NAA combined with BA/kinetin, the higher the callus percentage formation. Callus percentage formation in the media with the addition of BA/kinetin with NAA at a concentration of 0.5 mg/L was 60 and 70%, at a concentration of 1 mg/L NAA was 66 and 81%, respectively. In comparison, media with BA/kinetin and NAA 3 mg/L combined gave 78 and 91% callus percentage formation.

In addition to influencing the callus percentage formation, the combination of growth regulators cytokinins and auxin also affected the callus fresh weight (Fig. 3). Callus



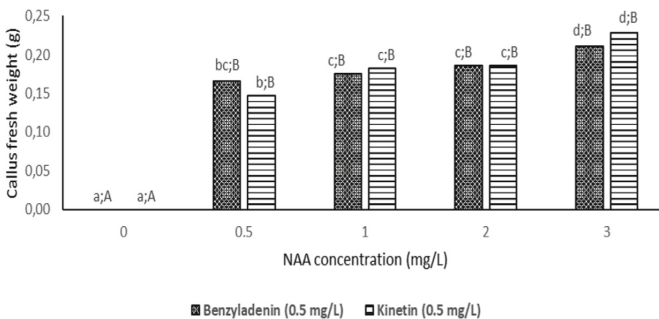
**Fig. 1.** Response of callus growth from leaf explants on the medium MS with the addition of plant growth regulator 0.5 mg/L BA/kinetin combined with several concentrations of NAA.



**Fig. 2.** The effect of auxin and cytokinin combination on callus percentage formation at week four of the culture period. Note: The different lowercase notation indicates a significant difference between different NAA concentrations, while the different uppercase notation indicates a significant difference between BA and kinetin (DMRT  $P < 0.05$ ).

growth on media with a combination of BA and NAA was not significantly different from callus growth on media with a combination of kinetin and NAA. The callus fresh weight on the combination of NAA and kinetin media ranged from 0.15–0.23 g, while the callus fresh weight on the combination of NAA and BA media was between 0.17–0.21 g. However, the callus fresh weight was significantly influenced by the concentration of NAA in combination with BA and kinetin. The higher the NAA concentration, the higher the wet weight of the callus. The callus fresh weight on media of BA/kinetin with NAA at a concentration of 0.5 mg/L was 0.17 and 0.15 g, at concentrations of 1–2 mg/L were almost the same at 0.18 and 0.19 g, while at a concentration of 3 mg/L NAA was 0.21 and 0.23 g. The combination of 3 mg/L NAA with 0.5 mg/L kinetin resulted in the highest callus fresh weight of 0.23 g.

Callus formation involves cell dedifferentiation, cell division and cell growth regulated by growth regulators, such as cytokinins and auxins, where the right combination of growth regulators will trigger callus cell proliferation and stimulate cell division [15]. The increase in endogenous growth regulators in cells can be caused by adding the



**Fig. 3.** Response of callus formation at week 4 of the culture period. Note: The different lowercase notation indicates a significant difference between different NAA concentrations, while the different uppercase notation indicates a significant difference between BA and kinetin (DMRT  $P < 0.05$ ).

hormone to the medium. The level of endogenous growth regulators has an important effect on physiological processes in explants, namely in controlling cell division [16]. An equal ratio of auxin and cytokinin will lead to callus induction [17]. However, each plant has different types and concentrations of appropriate growth regulators because they depend on plant tissues' (explants) genotype and physiological conditions [18] so that the determination of the composition of growth regulators to produce high callus requires an optimal composition of auxin and cytokinin [19].

In this study, patchouli leaf explants were cultured in 10 combinations of auxin and cytokinin to determine their effect on callus induction. Media with the addition of BA/kinetin without NAA (control) was not able to induce callus on leaf explants but instead induced shoots. A similar report in *Sida cordifolia* Linn, where explants grown on culture media containing cytokinin without auxin could produce earlier shoots [20]. When cytokinins are applied alone, the stimulatory or inhibitory effect on callus development depends on the concentration given. In this assume, auxin plays an essential role in callus induction [21]. In line, it can be seen from the results that callus formation depends on the presence of auxin and cytokinin together in the medium. These results are along with the research [22], which showed that callus induction with single growth regulators was less effective than a combination of auxin and cytokinin.

The percentage formation and fresh weight of callus were significantly influenced by NAA concentration, and the value increased with the increase in the concentration of NAA. The highest value was obtained at 3 mg/L NAA. This result indicates that the concentration of NAA influences callus formation. Similar results on the callus culture of *Catharanthus roseus* concluded that callus fresh weight and dry weight increased with increasing NAA concentrations [23]. The addition of NAA for callus induction will encourage cell division and enlargement [24]. With the increase in cell division, the availability of water in the cell also increases. The availability of more water will increase growth so that the wet weight also increases.

The media with NAA combined with kinetin could induce callus formation better than the combination of NAA with BA. Research [25] reported that kinetin positively affected callus where the percentage formation and fresh weight increased with increasing kinetin concentration. Previous studies have shown the use of kinetin to increase mitosis and cytokinesis in callus induction [26]. So that the increase in mitosis and cytokinesis can increase the callus percentage formation and callus fresh weight formed.

## 4 Conclusion

Patchouli callus can be induced from leaf explants cultured on MS media with a combination of BA and NAA or kinetin and NAA. The combination of kinetin with NAA induced callus formation better than the combination of BA with NAA. The combination of 0.5 mg/L kinetin and 3 mg/L NAA in the media resulted in the best callus percentage formation and callus fresh weight.

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