

THE EFFECTS OF FOLIAR AND NODES EXPLANTS ON CALLUS INDUCTION OF *POGOSTEMON CABLIN* IN-VITRO CULTURE BY USING PLANT GROWTH REGULATOR

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Abstract

Pogostemon cablin is a traditional herb that commonly found in South East Asian. In Malaysia, this plant is well known as “pokok nilam”. *Pogostemon cablin* has been used widely in medicinal and cosmetic products due to the high content of patchouli alcohol. However, disease-free plant of *Pogostemon cablin* which required to be used for industrial, can only possible to obtained via *in-vitro* culture. The used of plant growth regulators such as auxin and cytokinin are able to yield the desire result for this micropropagation outcome. Nevertheless, the types of explants used also have major possibility in creating variation towards end of cultured product. Therefore, in this research, most common explants which are foliar and nodes were used as main interest of this study in order to induce callus by using cytokinin and auxin. After one month of observation, both foliar and nodes explant successfully were able to initiate callus in combination of both auxin and cytokinin as early as 8 days after incubation. However, single hormone of auxin was only able to initiate callus after two weeks for both type of explants. The experiment later, had been preceded with callus propagation which were done by using the most optimum callus product. These calluses were selected as they fit the criteria of desirable callus which are soft, friable and whitish in coloration as to support the high performance of callus propagation rate that were successfully obtained.

Keywords: *Pogostemon cablin*, *In-vitro culture*, Callus Induction, Shoots Induction Explant Types, Plant Growth Regulators

Introduction

Pogostemon cablin is a traditional herb plant species that cultivated in tropical Asian region such as Malaysia and Indonesia. The local name for this plant is “Pokok nilam”. It is an erected perennial herb that belongs to family *Lamiaceae* and under the genus *Pogostemon*. It can grow up to 0.75 meter height under shade area with hot climate. The plant consists of bushy roots and produces small pinkish white flower that bearing tiny seeds. The stem is dark green colored when mature and light green during young. The blades leaves of *Pogostemon cablin* are dark green in color with oval shape and smooth surface about 2-10.5 × 1-8.5 cm broad (Hooker, 1983). The leaves of *P. cablin* are known to contain valuable properties of essential oil which give special odor even when it been rubbed. In natural plant, the chemical compositions of *P. cablin* are mostly oil alcohol bases such as phenol, aldehydes, and patchoulene, which are also called patchouli alcohol (Doneliana et al., 2009; Fatin and Khairiah, 2018). It is commonly used in cosmetic, pharmacology, and toxicology such as in commercialized products like

perfumes and hair conditioners. Study on callus and shoot induction of *P. cablin* can be highly beneficial towards the industrial usage of this plant due to disease-free plant clone. However, the use of different types of plant explants may exhibit the different potential outcome in term of external and internal morphology of culture product. In this study, two different types of above the ground plant organs were chosen as explants which were foliar and nodes. These explant were selected mainly due to low present of debris, foreign substance and soil evading bacteria which commonly causing the culture contamination. Apart from that, these explants also were easy to obtain and required lack of extensive care during explant collection compare to other plant organs. During the callus induction, the morphology of callus induced plays important role especially for somaclonal propagation for cloning process (Fatin et al., 2018). The desire callus outcome not only will reduce the propagation time but also will produce abundance clones products which later creating impact toward resources and actual yield especially for mass production (Kukreja et al., 1990). Therefore in this research, studied of the effect of two different types of explants were be measured in term of morphology of the culture products, coloration, texture, shape, frequency, abundance and also time needed for optimum callus induction by using plant growth regulators which were auxin and cytokinin.

Materials and Methods

Explants samples preparation and sterilization procedures

Approximately young foliar explants *P. cablin* of had been taken from natural plant and were around 3-4 mm² sizes. Meanwhile, the nodes of *P. cablin* were dissected around 3-5 cm length at the highest stem which has soft and green tissue that directly below the shoot tip area. The cut explants were prior wash under running tap water for debris removal. Then, the explants preceded for sterilization procedure by using ethanol 70%, 20% sodium hypochlorite (NaOCl) for 5 minutes. Next, the explants were rinsed with distilled water 3 times before continued had been cultured.

Media preparations

Basal media for callus induction was prepared by using Murashige and Skoog (1962). One liter of autoclave distilled water was added with 4.4 g/L of MS media powder, 30 g/L of sucrose, and 1g/L of Myo-inositol. Different concentrations of plant growth regulators were selected prior the series of pre-screening and were added in the media for main culturing process for callus induction (**Table 1**). The pH of the media as adjusted with high diluted of NaOH and HCl solution until it reached approximately \approx pH 5.8-6.8. Gelrite powder 8 g/l was added in each treatment and the media was autoclaved for 15 minutes in 121°C with 15 psi. The autoclaved media solution was cooled about 60°C to 50°C before was transferred into prepared petri dishes.

Table 1 Different concentration of plant growth regulator in MS media (mg/L) for callus induction of *P. cablin*

Treatment	Plant growth regulator concentration in mg/L
A	Control (absent of plant growth regulator)
B	0.25 mg/L of 2,4-D
C	0.5 mg/L of 2,4-D
D	0.5 mg/L of BAP and 3.0 mg/L of NAA

Callus Culturing Procedure and Data Collection

For callus induction, *P. cablin* foliar and nodes explants were used as samples. The sterilized explants were transferred into petri dish and samples were wrapped with the aluminum foil for dark condition and placed inside the growth chamber at temperature $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$. The data were taken after one month of observation period. Callus proliferation was done by sub culturing to determine the potential of high performance of callus from the prior optimum result in callus induction experiment. The callus had been transferred in petri dish which contained optimum media selected and rate of propagation of callus was observed within one month duration.

Result and Discussion

The experiment of callus induction was begun by culturing two types of explants which are nodes and foliar of *P. cablin* in series of pre-screening plant growth regulator treatment due its recalcitrant nature. Recalcitrant is the obstinate phase of explants cultures to undergo process of morphogenesis. This species had shown multiple occasions of non-successful product yield for *in-vitro* culture. After a few application of both and single combination of various plant growth regulator of auxin and cytokinin, the treatment with 0.25 mg/L of diclorophenoxyacetic acid (2,4-D), 0.5 mg/L of 2,4-D, 0.5 mg/L 6-benzylaminopurine (BAP) and 3.0 mg/L α -naphthaleneacetic acid (NAA) had shown positive result toward callus initiation and induction in both leaf and nodes explant.

Therefore, the replications for the data analysis were focussed on the effects of only three selected plant growth regulator concentrations on both foliar and nodes explant of *P. cablin* in callus induction. From the result, **Table 2** had showed that, all the selected treatment media were able to induce callus except control media in 30 days by using foliar explant (**Figure 1a**). The absence of callus indicates there was no cell differentiation had occurred, as the cell division had unsuccessfully been triggered without the present of optimum plant growth regulator as the catalyst for cell division (Hemashree et al., 2005).

However, even though the callus had initiated and successfully induced in other treatment, the morphology of callus quality was considered to be poor with dark brown coloration. The brownish color of callus may prone to cell death faster than the whitish color which may cause quite difficulty for the callus sub-culturing and propagation for future study. The treatment with single hormone 0.25 mg/L 2,4-D and 0.5 mg/L 2,4-D had produced very poor callus on day 20 and 18 respectively which not only dark brown coloration but also with of lack friability that caused callus to be hard and strain for propagation culture (**Figure 1b** and **1c**).

This selective double plant growth regulator treatment of 0.5 mg/L BA and 3.0 mg/L NAA was able to produce the earliest callus formation (day 8) with profuse brownish callus and soft callus texture. This slightly better result may had promising rate of successful callus propagation compare to other two due to high friability of callus that easier to sub culturing, high chances for nutrient absorption in media culture and also reduce the possibility of cell death (Arpana et al., 2008; Barbulova et al., 2014; Yu et al., 2017). The same selective hormone treatment was repeated by using nodes explants with unexpected different outcome. The nodes explants showed high totipotency cell compare to foliar explants with successfully callus induction in all treatment including control media.

The nodes cell showed the ability to profuse callus without the aid of plant growth regulator even though the callus morphology was relatively poor (**Table 3** and **Figure 2a**). The callus in MS media control may form due to secretion of endogenous growth regulator auxins such as Indoleacetic acid (IAA) or indole-3-acetamide (IAM), where IAM is the intermediate in auxins biosynthesis by cell (Wendy et al., 2009). This also indicates that the nodes cell has higher ability for the cell growth compare to foliar cell (Virendra et al., 2007; Santos et al., 2011; Yan

et al., 2015). The *P. cablin* nodes explants also exhibited desired result for callus by successfully induced high friable callus cell with white color morphology and soft texture in treatment 0.5 mg/L BA and 3.0 mg/L NAA. These criteria were required to sustain high chances of callus propagation in order to achieve optimum result which fast and abundance callus yield.

This probability had later been supported by observation of rate of callus propagation by sub-culturing this optimum callus in treatment 0.5 mg/L BA and 3.0 mg/L NAA in one month duration. After a month, the development of callus was observed in diameter (mm), which the size of the callus diameter (mm) has positive relationship with time in week, with regression line ($R^2 = 0.804$). The development callus has whitish color compared to its initial stage in which has darker color. The callus morphology appeared in clustered ball of globular cell instead of friable in cultured media (**Figure 3a**).

Table 2 Callus induction of *P. cablin* by using foliar explants in MS media with various plant growth regulators in one month *in-vitro* cultured

Plant hormone Concentration	Earliest Day of callus Formation (in days)	Percentage formation (%frequency)	Degree of callus induced	Callus morphology
Control	-	-	-	-
0.25 mg/L 2,4-D	20	66.7±0.33	+	Dark brown, hard, non-friable,
0.5 mg/L 2,4-D	18	86.7 ±0.28	++	Dark brown, hard, non-friable,
0.5 mg/L BAP and 3.0 mg/L NAA	8	100±0.0	++++	Dark brown, soft, friable,

Notes: Callus growth indicator; (-) = callus absent, (+) = very poor, (++) = poor, (+++) = average, (++++) = profuse

Table 3 Callus induction of *P.cablin* by using nodes explants in MS media in various plant growth regulators in one month *in-vitro* culture

Plant hormone Concentration	Earliest Day of callus Formation (in days)	Percentage formation (%frequency)	Degree of callus induced	Callus morphology
Control	22	46.7±0.27	+	Whitish brown, very hard non-friable
0.25 mg/L 2,4-D	17	56.8±0.32	++	Dark brown, hard, non-friable,
0.5 mg/L 2,4-D	11	90.0 ±0.37	+++	Dark brown, hard, non-friable,
0.5 mg/L BAP and 3.0 mg/L NAA	8	100±0.0	++++	Whitish brown, soft, high friable,

Notes: Callus growth indicator; (-) = callus absent, (+) = very poor, (++) = poor, (+++) = average, (++++)= profuse

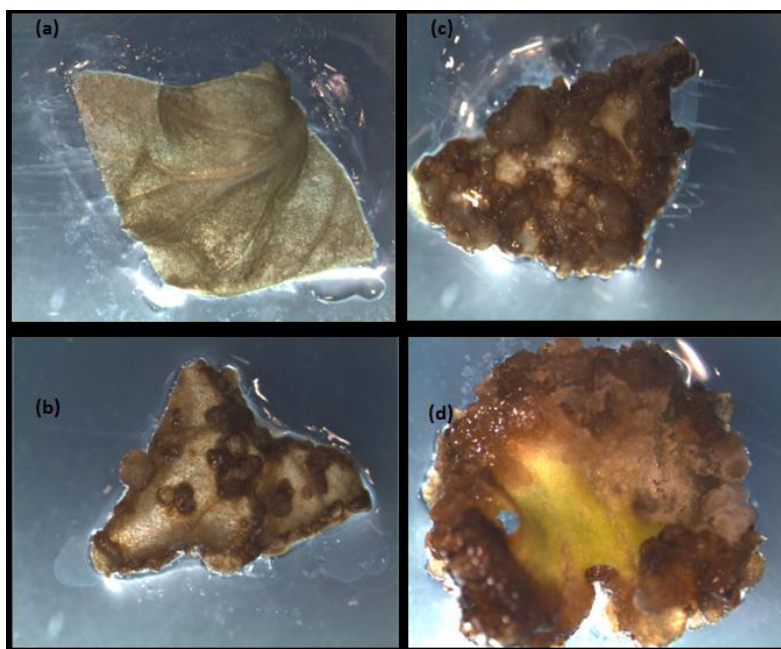


Figure 1 Callus induction of *P. cablin* leaf explants in MS media in one month. (a) Control, (b) 0.25 mg/L of 2,4-D, (c) 0.5 mg/L of 2,4-D, (d) 0.5 mg/L BAP and 3.0 mg/L NAA

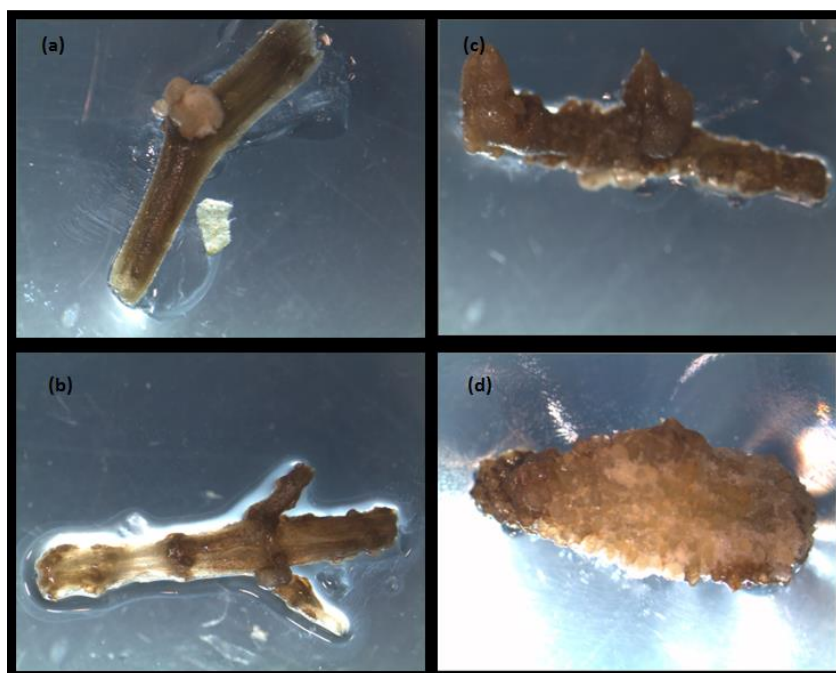


Figure 2 Callus induction of *P. cablin* nodes explants in MS media in one month. (a) Control, (b) 0.25mg/L of 2,4-D, (c) 0.5mg/L of 2,4-D, (d) 0.5mg/L BAP and 3.0mg/L NAA

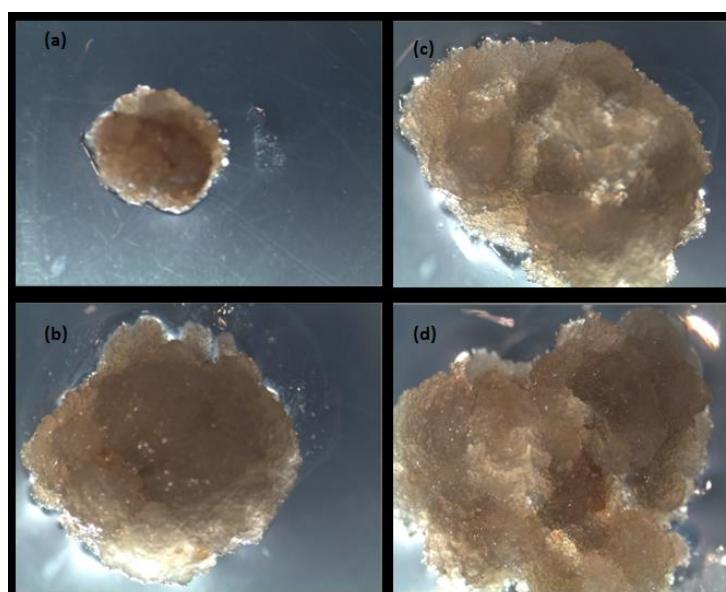


Figure 3 Rate of callus proliferation of *P. cablin* by using nodes explants in MS media supplement with 0.5 mg/L BAP and 3.0 mg/L NAA after one month (4 week). (a) 1st week (b) 2nd week, (c) 3rd week, (d) 4th week

Conclusion

Each plant organ of *P. cablin* has different ability to exhibit potential explants during *in-vitro* culture. This had been proven in this experiment where the used of both foliar and nodes explants had caused the different yield toward high performance callus products. With the aid of plant growth regulators which were auxin and cytokinin, both explants successfully induced calluses with different morphology. Based on the result, nodes explants had higher potential in producing optimum callus with desirable criteria which were whitish, soft and friable

morphology compare to foliar explants. The high growth rate potential of optimum callus were also successfully obtained after sub-culturing process in one month duration, where the callus showed positive increment of diameter which indicate rapid cell division with lack of cell death.

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