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Research Article

ENHANCING CALLUS FORMATION IN TAPAKTUAN PATCHOULI THROUGH *IN VITRO* OPTIMIZED COMBINATIONS OF PGR

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ARTICLE HIGLIGHTS

- Different varieties of Patchouli exhibited different growth pattern as well as *in vitro* culture
- In order to induce callus, it has been determined that the combination of regulators is necessary.
- Benzylaminopurine has been identified as a potential regulator that could be used to further develop the patchouli callus of Tapak Tuan.

Article Information

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ABSTRACT

Patchouli is known for its highly demanded essential oil. The Patchouli Tapaktuan variety is the most widely cultivated by local farmers in Aceh due to its high oil yield. Currently, the study and propagation of patchouli through a biotechnological approach is being developed, one of which is in vitro culture method. In this method, suitable Plant Growth Regulators (PGR) are being observed to enhance the growth of the explant. PGRs are synthetic compounds that are added to media in plant tissue culture to stimulate plant growth. This study aimed to analyze and optimize the effect of benzylaminopurine (BAP), thidiazuron (TDZ) combined with Naphthaleneacetic acid (NAA) on the growth of Tapaktuan patchouli leaf callus. This study was carried out by using a completely randomized design with seven treatments. Each treatment was replicated four times and each replicate contained three explants. The concentrations used were BAP 0.75 mg/L, TDZ 1 mg/L, and NAA in the 0.25 - 0.75 mg/L range. According to this study, combining BAP and NAA was the most effective for inducing callus in Tapaktuan patchouli leaves. The combination of BAP 0.75 mg/L + NAA 0.5 mg/L (treatment A2) was the most effective for inducing callus formation. This treatment resulted in the quickest callus development, the highest percentage of callus formation, and the largest callus diameter. Explants cultured with BAP produced a greenish-yellow callus having the potential for organogenesis culture, which could produce shoots having the ability for producing mass plantlets.

Keywords: *c*allus, *in vitro*, patchouli, PGR, Tapaktuan

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) is a fragrant, herbaceous plant that yields an essential oil called patchouli oil. Patchouli oil is used in the manufacture of perfumes, cosmetics, aromatherapy, and pesticides. Patchouli has long been used as a traditional remedy to treat various ailments, such as skin problems, insomnia, and as an aphrodisiac (Gopi 2017).

Patchouli is primarily produced in Sumatra and Sulawesi, with Aceh Province accounting for around 80% of global patchouli oil supply in 1980. Since 1921, Aceh has been the largest producer of patchouli oil (Ernawati *et al.* 2021). According to the Directorate General of Estates (2022) patchouli production in Indonesia has experienced a good increase, with a percentage increase of up to 60% from 2017 to 2021. Aceh has three high-yielding patchouli varieties with average oil production per hectare, which are Lhokseumawe (415.65 kg/ha), Sidikalang (464.442 kg/ha), and Tapaktuan (583.26 kg/ha). Of these three varieties, the Tapaktuan variety is the most widely cultivated

by local farmers due to its higher oil yields, more resilience to drought, and 10.6% more patchouli alcohol (PA) compared to the other two varieties. Patchouli alcohol, or PA, is a quality indicator for patchouli oil; the higher the PA value, the better the quality. Patchouli is usually propagated by cuttings, and it is quite difficult to obtain good-quality cuttings (Kandarihi *et al.* 2015).

The propagation of patchouli through a biotechnological approach in plant tissue culture has come a long way as the demand for patchouli oil increases every year (Swamy & Sinniah 2016). Using tissue culture, plants can be propagated rapidly and produce seedlings in large quantities. Plant propagation is achieved by selecting patchouli plants that are free from pests and diseases and using organ parts, such as shoots, nodes, and leaves, as sources of explants. Mass propagation in tissue culture can occur through somatic-embryogenesis or organogenesis. The formation of plant organs occurs in response to the wounding of plant tissues to form callus, the amorphous cells (Bidabadi & Jain 2020). New plants are formed from callus cells that have the capacity for embryogenic totipotency when supported by the use of suitable Plant Growth Regulators (PGR) (Fehér 2019).

The presence of PGRs added to the media influences the success rate of plant tissue culture (Fitroh *et al.* 2018). Auxin and cytokinin are types of PGR commonly used to induce callus in many plant species, and the explant growth rate depends on the concentration and combination of the two regulators, the species, and the type of explants (Hemmati *et al.* 2020). The amount of auxin and cytokinin administered to the explants determines the direction of plant explant growth. High cytokinin levels promote bud formation, high auxin levels encourage root development, and equal concentrations of both hormones lead to callus formation (Bano *et al.* 2022; Pulianmackal *et al.* 2014).

One type of auxin commonly used in tissue culture is Naphthaleneacetic-acid (NAA), which is often used to induce callus and roots (Kawochar *et al.* 2017). NAA concentration of 0.5-1.5 mg/L successfully induced callus of patchouli leaves of local Indian varieties with an average formation percentage of 94.34% within 2-4 weeks of culture of the explants (Lalthafamkimi *et al.* 2022).

NAA is also often combined with cytokinin, such as benzylaminopurine (BAP) to promote the formation of green callus that can develop into shoots. Florenika *et al.* (2022), found that NAA 0.5 mg/L + BAP 0.1 mg/L was the best combination to produce morphogenic callus in Sidikalang variety patchouli, while in local Indian patchouli, the highest morphogenic callus of 81.51% was formed in the combination of NAA 1.5 mg/L + BAP 1.5 mg/L (Lalthafamkimi *et al.* 2022).

In addition to BAP, a regulator categorized as cytokinin, thidiazuron (TDZ), is also frequently used for plant cell proliferation and regeneration. TDZ combined with NAA gives superior results than those combined with other types of auxins. In the morphogenesis of *Scutellaria bornmuelleri* and *Ajuga bracteosa* plants, the concentration of TDZ ranges of 0.5 - 1.2 mg/L giving a higher response to explant morphogenesis (Ali *et al.* 2018; Gharari *et al.* 2021). The studies conducted by Tarigholizadeh *et al.* (2021) on *Satureja sahendica* Bornm had the highest percentage of callogenesis (95.24 - 100%) at different levels of NAA and TDZ combination treatments (0.5 - 2.0 mg/L).

To date, research on PGRs for the induction of Tapaktuan patchouli leaf callus has not been fully conducted. This is because each varieties of patchouli have particular superiority and physiological responses. Callus induction is utilized for obtaining germplasm from a small source, which can then be studied in advanced biotechnology applications, such as genetic engineering and secondary metabolite engineering.

Florenika *et al.* (2022) investigated the most effective combination of PGRs for inducing callus in Sidikalang variety patchouli leaves, which is BAP + NAA. In the Lhokseumawe variety of patchouli, Puspita *et al.* (2023), obtained optimal results for inducing leaf callus in the combination of TDZ 0.75 mg/L + NAA 0.5 mg/L and BAP 0.75 mg/L + NAA 1.0 mg/L. Therefore, from these studies, it is necessary to conduct research for determining the best combination of PGRs in order to induce callus in patchouli leaf explants of the Tapaktuan variety. This study aimed to analyze and optimize the combination of PGRs BAP, TDZ, and NAA on the growth, development, and callus morphology of Tapaktuan patchouli leaf explants.

MATERIALS AND METHODS

Plant Preparation

Patchouli seedlings of the Tapaktuan variety were obtained from Nino Park, Syiah Kuala University, Banda Aceh. The seedlings were quarantined by spraying them with 2 g/L bactericide (active ingredient of Streptomycin sulfate 20wp) and 2 g/L fungicide (active ingredient of benomyl 50wp) separately every two days for two to three weeks and fertilized once a week with 80 g of 16-16-16 (NPK) fertilizer to stimulate leaf growth.

Explant Sterilization

Sterilization was carried out outside and inside the Laminar air flow cabinet (LAFC). Sterilization outside the LAFC is based on Puspita *et al.* (2023), where leaves of quarantined plants were rinsed under running water and carefully brushed with a soft brush until clean. The explants were first soaked in 5% detergent for 5 minutes and washed again under running water until clean. Next, the explants were soaked in 2% bactericide for 15 minutes, agitated every 5 minutes, and rinsed three times with sterile water. The explants were then immersed in a 2% fungicide for 15 minutes, agitated once every 5 minutes, and rinsed three times with sterile water.

Following the sterilization inside the LAFC as described by Rahmawati *et al.* (2021), the explants were transferred to the LAFC and further sterilized for 5 minutes with a 30% NaOCl solution containing a drop of Tween 80, then rinsed three times with sterile water. Finally, the leaves were immersed in ethanol 70% for 30 seconds and then rinsed three times with sterile water.

Culture Medium

Sterilized leaf explants were placed into a petri dish and dried using sterile tissue. The explants were cut into smaller 1 cm x 0.5 cm pieces with a scalpel. The explants were cultivated in Murashige & Skoog (MS) medium, which contained 30% sucrose, 5.5% agar, NAA (0.25; 0.50; and 0.75 mg/L), BAP (0.75 mg/L), and TDZ (0.75 mg/L). For comparison, the explants were cultured into MS without any addition of PGR (MS0), as shown in Table 1. The explants were then incubated at 22 °C to 24 °C under a 16-watt fluorescent lamp (Philips T8 Ecofit cool daylight), 10 cm away from the lamp and the explant bottle.

Explant growth was observed for 60 days, as the patchouli callus had not fully developed after 30 days, and to gain a clear visualization of the callus from the effect of each treatment. The growth of the explant was monitored every day after the explants were cultivated.

Data Analysis

A completely randomized design was used in this study with 7 treatments. Each treatment was replicated four times and each replicate contained three explants. The combination of treatments can be seen in Table 1.

The data of the individual parameters were calculated as a percentage. The callus response of the patchouli variety Tapaktuan was determined using variables, such as percentage of callus, percentage of live explants, percentage of shoots, percentage of roots, and callus diameter. Callus formation was observed daily until callus began to emerge from the edge of the explants.

Table 1 Comnbination of plant growth regulators used in the study for callus induction of *P. cablin*

Treatments	Plant Growth Regulators (PGR) (mg/L)		
	BAP	TDZ	NAA
MS0	-	-	-
A1	0.75	-	0.25
A2	0.75	-	0.50
A3	0.75	-	0.75
A4	-	1.0	0.25
A5	-	1.0	0.50
A6	-	1.0	0.75

The percentage of callus formation was observed on day 60 after initiation (DAI) and live explant parameters were taken on days 30 and 60 of observation to determine the ability of the callus to survive in the treatment. The formula used was based on Wulandari *et al.* (2022):

$$\% \text{ Callus} = \frac{\textit{Number of explants that form callus in each treatment}}{\textit{Total of all explants planted}} \text{ x } 100\% \quad (1)$$

$$\% \text{ Live Explant} = \frac{\textit{Number of living explants per treatment}}{\textit{Total of all explants planted}} \text{ x } 100\% \quad (2)$$

The diameter of the callus explants was measured on day 60 after the start. Data were collected by measuring the width of the explants that produced callus at the bottom of the explant bottles using a ruler. The diameter of callus explants was determined using the formula on Taghizadeh *et al.* (2020):

$$\text{Callus diameter} = \sqrt{\textit{(Callus length x callus width)}} \quad (3)$$

Explants that formed shoots and roots were observed by counting the number of explants planted in each treatment that showed signs of the emergence of shoots and roots. The percentage of shoots and roots formed was calculated based on Fanata and Qudsiyah (2020), using the formula:

$$\% \text{ Shoots} = \frac{\textit{Number of explants that form shoots per treatment}}{\textit{Total of all explants planted}} \text{ x } 100\% \quad (4)$$

$$\% \text{ Roots} = \frac{\textit{Number of explants that form roots per treatment}}{\textit{Total of all explants planted}} \text{ x } 100\% \quad (5)$$

The callus morphology, including color and texture, was observed visually. The color of the explant callus was observed on day 60 DAI. Callus color was visually observed and determined using the Munsell Color Chart method by Restanto *et al.* (2022), followed by a callus color scoring method based on Thomy (2012), shown in Table 2.

Explant texture observations were made visually and by touching with tweezers. Rasud and Bustaman (2020), define a friable texture as one that is highly soft, quickly disintegrates, and slightly sticky to the tweezers. A compact callus feels very dense and firm, while an explant with both textures is considered intermediate.

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Table 2 Callus color score

Score	Callus color
1	Brown
2	Brownish white
3	Brownish green
4	Yellowish white
5	Yellowish green
6	Whitish green
7	Green

RESULTS AND DISCUSSION

Days of Callus Formation

The appearance of callus in explants of patchouli leaves is characterized by the fact that the explants bend and swell two weeks after cultivation. The callus grows from the injured tissue and gradually spreads around the injured area and surface of the explant until it covers all parts. According to Habibah *et al.* (2021), the involvement of regulators in culture media has a significant impact on the development of calluses. In this study, BAP and TDZ together with NAA were able to induce callus on patchouli leaves of the Tapaktuan variety. The ability of TDZ and BAP on the day of callus formation was significantly different from each other. The results of the ANOVA test results ($P < 0.05$) indicated that there was a significant difference on the day of callus formation from the combination of BAP, TDZ, and NAA. However, the callus could not appear in MS0 treatment until 60 days of observation.

Callus appeared on day 15 in the treatment of BAP 0.75 mg/L combined with NAA 0.25 mg/L (A1) and NAA 0.5 mg/L (A2), which is similar to research results of Puspita *et al.* (2023), where the callus of patchouli leaf explants of the Lhokseumawe variety in the combination of BAP 0.75 mg/L + NAA 0.50 mg/L showed signs of callus appearance on day 15 day of observation. Callus grown with TDZ 1.0 mg/L + NAA 0.25 mg/L successfully induced the quickest callus on day 16 of observation. BAP combined with NAA successfully induced callus in *Nepeta binaloudensis* Jamzad and cucumber (*Cucumis sativus* L.) leaf explants (Faisal *et al.* 2018; Sagharyan *et al.* 2020).

Exogenous cytokinin, such as BAP and NAA, have been shown in in vitro culture of many plant species to induce callus and promote cell division. According to previous studies, there is a balance of auxin and cytokinin that work together to stimulate cell division, which is a crucial phase in callus development (Sagharyan *et al.* 2020). According to Deswiniyanti and Lestari (2020), exogenous auxin, such as NAA, increases osmotic pressure, elongation, and cell enlargement, whereas cytokinin, such as BAP promotes cell division. Auxin increases water diffusion into cells, hence the combination of auxin and cytokinin stimulates cell division and modulates cell differentiation pathways. Combining TDZ with other phytohormones is highly recommended because TDZ might have significant effects on the metabolism of other phytohormones through accumulation and translocation (Deepa *et al.* 2018). In the present research, the treatment of TDZ 1.0 mg/L combined with NAA 0.25 mg/L resulted in the fastest callus on day 16, compared with other TDZ combinations.

Callus Formation Percentage and Morphological Development

The formation of callus on cultivated explants is an indicator of the growth reaction of the explant. In leaf explants, callus appears first on the leaf bones because the leaf veins have vascular bundles that transmit nutrients to all sections of the leaf surface, allowing the cells near the leaf veins to divide and induce callus (Rahayu & Mardini 2015). The results of this research found that the patchouli leaf callus cultivated under all treatments showed callus formation (Table 3).

Table 3 Effect of PGR on the growth of *P. cablin* callus explant Tapaktuan variety

PGR	Callus formation (day)	Callus %	Live explants %	Callus diameter (mm)	Shoots (%)	Roots (%)
MS0	60 ± 0^{d}	0.0 ± 0^{b}	0.0 ± 0^{c}	0.0 ± 0^{a}	0	0
A1	15 ± 0.49^{a}	**100 ± 0^{a}**	**100 ± 0^{a}**	164.4 ± 14.4^{b}	66.7 ± 2.3	0
A2	**15 ± 0.37^{a}**	**100 ± 0^{a}**	91.7 ± 8.3^{a}	**187.9 ± 8.70^{b}**	83.4 ± 2.5	0
A3	16 ± 1.05^{ab}	91.7 ± 8.3^{a}	75.0 ± 16.0^{a}	177.5 ± 17.0^{b}	0	0
A4	16 ± 0.56^{a}	86.7 ± 8.2^{a}	86.7 ± 8.2^{a}	166.1 ± 22.9^{b}	0	83.4 ± 2.5
A5	20 ± 0.76^{c}	91.7 ± 8.3^{a}	91.7 ± 8.3^{ab}	153.3 ± 16.4^{b}	0	100 ± 2.3
A6	18 ± 1.01^{bc}	**100 ± 0^{a}**	83.3 ± 16.7^{b}	137.6 ± 27.2^{b}	0	33.3 ± 1.3

The A1, A2, and A6 treatments were the combination that resulted in the callus formation with the highest percentage (100%) followed by the A3 and A5 treatments (91.7%). The A4 treatment produced the lowest callus formation percentage (86.7%). Patchouli leaf explants in the MS0 treatment did not form any calluses.

In the treatment of BAP combined with NAA 0.25 mg/L and 0.5 mg/L, callus was successfully induced at a 100% rate (Table 3). This is in accordance with the results reported by Anwar and Isda (2021), in which the addition of high concentrations of BAP combined with low concentrations of NAA gave the best results in the formation of *Centella asiatica* L. callus, with a 100% formation rate in the treatment combination of BAP 1 mg/L + NAA 0.3 mg/L. However, when BAP and NAA were administered at concentrations near to, equal to, or greater than 1.0 mg/L, the percentage of callus formation started to decline, as observed in treatment A3, which achieved 91.7% callus formation (Table 3). This result is similar to the result of Nazir *et al.* (2020), the combination of BAP 2 mg/L + NAA 1 mg/L resulted in 92% callus formation in *Ocimum basilicum* explants.

In TDZ, treatment A4 induced 86.7% of calluses, which increased to 91.7% in treatment A5. Then, in treatment A6, the callus production rate reached 100%. The percentage of callus increases with higher concentrations of NAA and TDZ, indicating a positive trend in callus production on TDZ treatments. Puspita *et al.* (2023), reported that patchouli leaf explants of the Lhokseumawe variety treated with TDZ 1.0 mg/L + NAA 0.5 mg/L made the highest percentage of callus formation (66%).

Explants cultivated in medium containing a combination of BAP and NAA produced Yellowish-green callus, meanwhile explants cultivated in medium containing TDZ and NAA produced brownish-green callus (Fig. 1). All combination treatments produced compact-texture callus (Table 4).

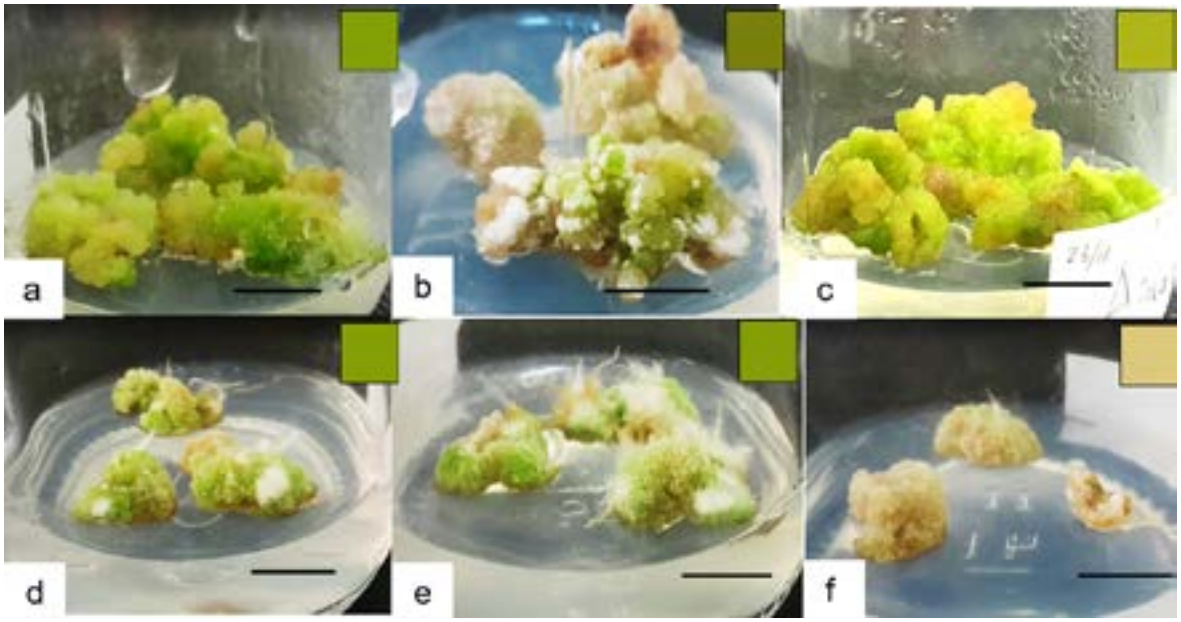

Figure 1 Patchouli leaf callus at 8 weeks after initiation

Notes: a) BAP 0.75 mg/L + NAA 0.25 mg/L treatment; b) BAP 0.75 mg/L + NAA 0.5 mg/L treatment; c) BAP 0.75 mg/L + NAA 0.75 mg/L treatment; d) TDZ 1.0 mg/L + NAA 0.25 mg/L treatment; e) TDZ 1.0 mg/L + NAA 0.5 mg/L treatment; f) TDZ 1.0 mg/L + NAA 0.75 mg/L treatment; Scale bar = 1 cm; the colored box on the top right shows the visualization of callus color using Munsell color chart.

Table 4 Callus morphology

Treatment	Callus color		Texture
	Munsell code	Score	
MS0	-	-	Compact
A1	5 GY 6/10 (Yellowish green)	5	Compact
A2	2.5 GY 5/8 (Yellowish white)	**6**	Compact
A3	2.5 GY 7/10 (Yellowish green)	5	Compact
A4	5 GY 6/10 (Brownish green)	3	Compact
A5	5 GY 6/10 (Brownish green)	3	Compact
A6	7.5 Y 8/6 (Brown)	1	Compact

Source: Munsell (1977); Thomy (2012).

The callus color reflects its visual appearance as an indicator of whether the callus contains cells that are actively dividing, embryonic, or cells that have undergone senescence. Green and yellow callus have a good ability to divide. Green callus is due to the presence of chlorophyll in the callus. A yellow callus indicates the presence of flavonoid pigments. Brown callus indicates the metabolism of phenolic compounds which are toxic and can inhibit plant growth, potentially leading to cell death (Tarigan *et al.* 2023; Wijaya *et al.* 2023). Callus in treatment A2 also produced white callus, which according to Tarigan *et al.* (2023), shows that it has embryogenic potential, allowing it to develop into somatic embryos.

Live Explant Percentage

The percentage of live explants indicates their ability to respond to the treatment of regulators to induce callus formation. This study found that combining BAP and TDZ with NAA significantly increased the percentage of live explants ($P < 0.05$). According to the results shown in Table 3, treatment A1 produces the maximum percentage of live explants by 100% and has the highest percentage of live explants in the combination of BAP. Explants cultivated on TDZ had the highest percentage of live explants (91.7%) in the A5 treatment. Surviving explants have fresh, light-colored explants with a callus that continues to differentiate and proliferate. According to Waryastuti *et al.* (2017), the callus that does not survive turns a blackish-brown and shows no signs of cell growth.

When the concentration of NAA was raised to 0.50 mg/L (A2), the percentage of live explants began to decrease to 91.7%; when the concentrations of BAP and NAA were equal at 0.75 mg/L (A3), the percentage of live explants decreased to 75%. Callus cultivated in TDZ decreased in percentage by 83.3% when combined with NAA 0.75 mg/L (A6). This might be due to the mechanism of hormone inhibition by other hormones (Septiana 2014). Aside from the exogenous hormones administered, the explants produce their own endogenous hormones, one of which is IAA (indole acetic acid) (Korasick *et al.* 2013). According to Septiana (2014), high levels of auxin may inhibit tissue growth due to competition with endogenous auxin for a signal receiver position in the cell membrane, resulting in inhibition of cell growth and elongation. If endogenous IAA levels increase, this type of auxin might lower the efficiency of BAP.

Callus Diameter

The diameter of the callus indicates the explants' response in the form of callus growth to the applied regulators. As shown in Table 3, the largest callus diameter in BAP treatment was obtained in the A2 treatment (BAP 0.75 mg/L + NAA 0.50 mg/L), with an average callus diameter of 187.9 mm. The maximum average callus diameter in the TDZ treatment was 166.1 mm in treatment A4 (TDZ 1.0 mg/L + NAA 0.25 mg/L). Meanwhile, the MS0 treatment did not form any calluses, therefore, its diameter could not be measured. Explants cultivated under BAP treatment fluctuated in response to increasing NAA concentrations. Studies on *Dracocephalum polychaetum* and *Dracocephalum kotschyi* leaf callus explants similarly showed variations upon increasing NAA from 0.5 mg/L to 1.0 mg/L (Taghizadeh *et al.* 2020). In the TDZ treatment,

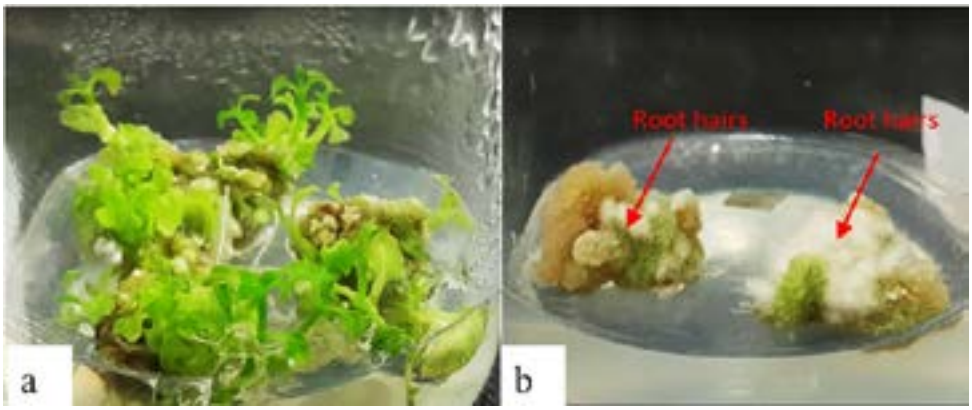

Figure 2 a) callus with shoots in BAP 0.75 mg/L + NAA 0.5 mg/L treatment. b) callus with roots TDZ 1.0 mg/L + NAA 0.5 mg/L treatment

callus diameter decreased as NAA concentration increased. This result is similar to the callus of *Mentha pulegium* L., which decreased with increasing NAA concentration when combined with benzyladenin (Jafari *et al.* 2016).

The size of the callus produced varies depending on the plant tissue's ability to absorb water and nutrients. Generally, auxin stimulates callus formation, while cytokinin can also enhance callus formation, although its specific mechanism is unknown. According to some researchers, genetics have a crucial role in forming callus in tissue culture and the regeneration capability of plants, which are controlled by numerous genes, particularly in the nucleus and cytoplasm (Gharari *et al.* 2021).

Auxin and cytokinin function together to promote callus development in explants, as there is a synergistic and antagonistic interaction between auxin and cytokinin. The use of high amounts of cytokinin with low amounts of auxin can synergistically trigger cell division and regeneration in vitro (Faisal *et al.* 2018). Auxin can antagonistically inhibit cytokinin biosynthesis signaling pathways, while cytokinin promotes auxin biosynthesis and regulates auxin transport to sites of differentiation and growth. However, an excess of cytokinin can have negative effects on plant growth (Kotov & Kotova 2023). The combination of higher auxin with cytokinin may slightly suppress the inhibitory effect of auxin and the negative effects of cytokinin. Some researchers speculate that auxin, which is synthesized with the help of cytokinin, acts as a feedback loop that inhibits the accumulation and activity of cytokinin (Kurepa & Smalle 2022).

Root and Shoot Formation

The presence of PGR, specifically auxins and cytokinin, has a strong influence on callus growth and development. The morphogenesis of explants always depends on the interaction between auxin and cytokinin. The concentration of auxin and cytokinin regulates the amount and direction of growth in the callus and organogenesis. Some explants treated with BAP formed shoots from calluses, which formed 0.5 - 1 cm shoot. Shoots are formed in treatment A1 and A2 with the highest percentage of 83.35% in treatment A2 (Table 3; Fig. 2a). Explants treated with TDZ, which was treatment A4 to A6 (Table 3), did not produce shoots, instead appeared root-like in the form of white, fine root hairs (Fig. 2b).

Treatment A5 had the highest percentage of roots at 100%. Although TDZ is also a cytokinin, the callus formed root hairs rather than shoots, which contradicts the concept of organogenesis by auxins and cytokinin. A higher auxin concentration in comparison to cytokinin results in the production of a rooted callus, whereas a high cytokinin content results in the formation of a shoot-like callus. When the given concentration of cytokinin and auxin is balanced or nearly balanced, the explants maintain the callus form (Sudrajad & Wijaya 2019). In addition to auxin and cytokinin, which are administered as exogenous hormones, the endogenous hormones within the explants also have an influence (Silalahi 2015). This could explain why the TDZ treatment of the callus produces roots rather than shoots.

Aside from the roots, auxin levels are often high in buds and shoots. If the explants used are very young leaves, the auxin content may still be quite high. Therefore, when the explants are cultured

in a combination of high cytokinin with low auxin media, endogenous auxin will most likely work together with exogenous auxin to lower the cytokinin content and suppress endogenous cytokinin biosynthesis (Sosnowski *et al.* 2023). According to Zhang *et al.* (2018), root hairs emerge in root epidermal cells and have several types of initiation patterns. In patchouli leaf explant callus, root hair initiation might occur through the differentiation of epidermal cells that produce roots at random. This pattern is commonly found in most dicotyledonous, monocotyledonous, and fern plants.

CONCLUSION

Callus initiation media containing cytokinin and auxin were reported to effectively grow the callus of Tapaktuan variety patchouli leaves. The use of PGRs could have different effects on plants of different cultivars within the same species. The relationship between PGRs and plant varieties within a species is not well understood and requires extensive research. Optimization research offers the best option for growing plants of specific varieties through tissue culture techniques. According to this study, combining BAP and NAA is most effective for inducing callus in Tapaktuan patchouli leaves. BAP 0.75 mg/L + NAA 0.5 mg/L (treatment A2) is the most efficient combination to induce callus Tapaktuan variety patchouli leaves. This combination resulted in the fastest duration, the highest percentage of callus formation, and the largest callus diameter. Explants in treatment A2 produced a yellowish-white callus with potential for organogenesis culture, capable of generating shoots that can produce mass plantlets.

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