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Comparative Evaluation of Cytokinin-Induced In Vitro Shoot Multiplication in Black Ginger (*Kaempferia paviflora* Wall. ex Baker)

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ABSTRAK

Kencur hitam (*Kaempferia paviflora* Wall. Ex Baker) Family Zingiberaceae dari Genus Kaempferia (Kencur-kencuran). Rimpangnya berwarna ungu gelap hingga kehitaman sebagai sumber senyawa bioaktif. Produksi bibit *K. parviflora* sangat terbatas karena masih secara secara konvensional. Tujuan penelitian untuk mengetahui jenis hormon sitokinin paling baik dalam multiplikasi kencur hitam (*Kaempferia parviflora*) secara in vitro. Eksplan yang digunakan eksplan in vitro *K. parviflora*. Media berupa Media Murashige and Skoog (MS) dengan perlakuan P0 (tanpa hormon), P1 (1 mg/l BAP), P2 (2 mg/l BAP), P3 (1 mg/l Kinetin), P4 (2 mg/l Kinetin), P5 (1 mg/l TDZ) , P6 (2 mg/l TDZ) dengan 10 kali ulangan. Parameter yang diamati yaitu persentase eksplan hidup, jumlah tunas, jumlah daun dan panjang tunas. Hasil penelitian bahwa eksplan hidup 94%, perlakuan P6 (TDZ = 2 mg/L) menghasilkan rata-rata jumlah tunas (4,9 tunas) dan rata-rata jumlah daun terbanyak (9,3 helai). Hal ini disebabkan oleh tingginya efektivitas TDZ konsentrasi rendah dibandingkan dengan sitokinin lainnya.

Kata Kunci : *Kaempferia paviflora*; Kencur hitam; Multiplikasi; Sitokinin

ABSTRACT

Kaempferia parviflora Wall. ex Baker (Zingiberaceae), commonly known as black ginger, produces dark purple to black rhizomes rich in bioactive compounds. Conventional propagation of *K. parviflora* is constrained by limited planting material availability. This study evaluated the effectiveness of various cytokinins for in vitro shoot multiplication of *K. parviflora*. Nodal explants derived from sterile in vitro stock were cultured on Murashige & Skoog medium supplemented with one of seven treatments: P_0 (no cytokinin), P_1 (1 mg L^{-1} BAP), P_2 (2 mg L^{-1} BAP), P_3 (1 mg L^{-1} kinetin), P_4 (2 mg L^{-1} kinetin), P_5 (1 mg L^{-1} TDZ), or P_6 (2 mg L^{-1} TDZ), with ten replicates per treatment. Parameters recorded after eight weeks included explant survival percentage, shoot number, leaf number, and shoot length. Explant survival averaged 94 % across treatments. Treatment P_6 (2 mg L^{-1} TDZ) yielded the highest mean shoot number (4.9 ± 2.6 shoots) and leaf number (9.3 ± 2.2 leaves) per explant. The superior performance of low-concentration TDZ compared to BAP and kinetin shows its high efficacy in promoting cytokinin-mediated shoot proliferation.

Keywords : *Kaempferia paviflora*; Black Ginger; Multiplication; Cytokinin.

I. INTRODUCTION

Kaempferia parviflora (black ginger) is a monocotyledonous plant native to Thailand and also distributed in Laos, Myanmar, India, China, and Indonesia (Park et al., 2021; Putiyanan et al., 2008). Unlike Curcuma caesia Roxb (commonly known as black turmeric), which belongs to the genus Curcuma and exhibits a less intense rhizome coloration and distinct morphological traits, K. parviflora possesses rhizomes that range from deep purple to black and are rich in bioactive compounds such as essential oils, phenolic glycosides, and a variety of flavonoids (Kitwetchar et al., 2020 ; Nuraeni et al., 2023). According to (Park et al., 2021) K. parviflora may serve as a valuable alternative source for the extraction of bioactive constituents. Traditionally, its rhizomes have been employed to treat colic disorders, general debility, hyperglycemia, male impotence, and ulcers. Pharmacological studies have demonstrated that K. parviflora rhizome extracts exhibit antioxidant, anti-allergic, anticancer, antimicrobial, anticholinesterase, anti-inflammatory, anti-obesity, and antimutagenic activities. Phytochemical analyses have confirmed the presence of flavonoids, methoxylated flavones, phenolic glycosides, and terpenoids (Rahman et al., 2018; Park et al., 2021; Trisombon, 2009; Tewtrakul & Subhadhirasakul, 2008; Vichitphan et al., 2007; Wattanatho et al., 2007: Picheansoonthon & Koonterm, 2008).Conventional propagation of K. parviflora is hampered by prolonged growth cycles, extended dormancy periods, and the limited availability of parental rhizome planting material. Planting stock is typically obtained from rhizomes harvested in the previous cropping cycle, with a maturation period of eight months (Zulfa, 2012) to as long as 12–14 months. Following harvest, rhizomes require a dormancy phase of 50–55 days (Karim et al., 2014; Khairudin et al., 2020) comparable to the 2–3 month dormancy observed in K. galanga (Rostiana & Effendi, 2007; Labrooy et al., 2020). These constraints not only limit seedling availability but also render the crop vulnerable to bacterial wilt caused by Ralstonia solanacearum and root-knot nematode (Meloidogyne spp.) infections, which lead to rapid rhizome decay (Zulfa, 2012). Consequently, K. parviflora cultivation remains heavily dependent on the yield of previous seasons.Given the growing demand for ginger species in the pharmaceutical industry (both domestically and internationally), the balance between raw material supply and planting material production must be addressed. Total exports of medicinal plant products (including dried plants, extracts, and finished goods) increased from 274,609.8 tonnes in 2022 to 289,390.3 tonnes in 2023 (Badan Pusat Statistika Indonesia, 2023; INATRIMS, 2014). In contrast, domestic production of ginger declined from 54,048,609 tonnes in 2021 to 52,477,225 tonnes in 2022, and further to 47,890,390 tonnes in 2023 (Badan Pusat Statistika Indonesia, 2024). To meet escalating industrial demands, a sustainable source of healthy, high-volume planting material is required. This is an objective unattainable by conventional rhizome propagation alone. Tissue culture biotechnology therefore offers an alternative solution

1. Problem Formulation

Plant tissue culture entails the aseptic cultivation of plant cells, tissues, or organs on nutrient-enriched artificial media to regenerate whole plants under laboratory conditions (Dwiyani, 2015). This technique has been proven to facilitate the rapid clonal multiplication of large numbers of genetically uniform plants in commercial nurseries.

2. Research Goal

In vitro culture is important in achieving outcomes that are not feasible via ex vitro (conventional) methods. One common approach to in vitro propagation is shoot multiplication (Siregar, 2022).

3. Research value

Cytokinins (adenine derivatives) are indispensable regulators of cell division and morphogenesis (Lestari, 2008). According to Asra dkk (2020), cytokinins function to: (1) promote cytokinesis (cell division); (2) stimulate overall plant growth; (3) induce seed germination; (4) influence root differentiation and growth; and (5) delay senescence. Because of the roles in cell division and shoot proliferation, cytokinins are widely used in shoot multiplication protocols (Nazhira et al., 2024). The present study aims to evaluate the effects of various cytokinins on the in vitro shoot multiplication of *K. parviflora*

II. RESEARCH METHOD

Perawatan post partum berfokus pada masalah yang berkaitan dengan ibu dan bayi sejak lahir hingga 6 minggu. Tujuan perawatan selama periode postnatal awal adalah untuk mempromosikan fisik kesejahteraan ibu dan bayi, serta menciptkan bonding attachment antara bayi dan orang tuanya juga keluarga (Purwaningsih, A, 2023). Selain itu, juga dapat mendukung pengembangan keterampilan menyusui bayi dan memperkuat pengetahuan dan kepercayaan ibu terhadap kesehatan dan kesejahteraan dirinya dan bayinya. Dengan demikian, pengetahuan perawatan pasca melahirkan memungkinkan ibu untuk mengembangkan keterampilan pengasuhan yang harus dipenuhi untuk menimbulkan peran keibuan mereka dalam keluarga. Hal ini sejalan dengan teori, dikarenakan Ny"A" Post op SC dengan indikasi fetal hipoksia dengan *absent end diastolic* dan IUGR.

III. RESULT AND DISCUSSION

Based on observations over an eight-week culture period, the survival rate of *Kaempferia parviflora* explants was 94 %, while 6 % of cultures were lost to fungal contamination. This is likely caused by suboptimal sterilization during initiation. The remaining 94 % of explants remained sterile and exhibited vigorous growth, with no instances of chlorosis or necrosis, due to the use of previously established, axenic in vitro stock (Figure 1). Figure 2 summarizes the weekly development, and Table 1 presents the mean values for shoot and leaf production across all treatments.

Table 1. Mean shoot and leaf counts per explant after eight weeks of culture (mean ± SD)

Treatment	**Mean counts per treatment**	
	Mean shoots per Explant	**Mean leaves per Explant**
P0	1,3 ± 0,7	3,5 ± 1,7
P1	3,4 ± 2,6	8,8 ± 5,3
P2	2,5 ± 1,6	5,2 ± 3,0
P3	2,9 ± 2,0	8,1 ± 4,8
P4	1,3 ± 0,8	3,9 ± 2,8
P5	3,2 ± 1,7	7,0 ± 4,3
P6	**4,9 ± 2,6**	**9,3 ± 2,2**

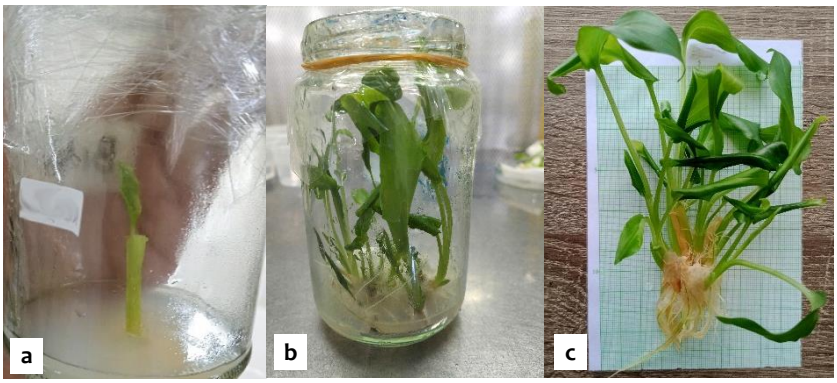

Figure 1. (a) Explant at 1 week after culture (1 WAC); (b) Explant at 4 WAC (1 month); (c) Explant at 8 WAC.

Figure 2. Explant removal and dissection for parameter measurement.

Number of Shoots

Adventitious shoots emerging from each nodal explant were counted at the conclusion of the eight-week culture period (8 WAC). As shown in Table 1, the highest mean shoot multiplication was obtained under treatment P_6 (2 mg L^{-1} TDZ), with 4.9 ± 2.6 shoots per explant, exceeding those observed with BAP and kinetin. Cytokinins are critical regulators of shoot induction, influencing cellular metabolism and activating dormant meristematic cells by promoting cell division (Jannah et al., 2022). Thidiazuron (TDZ) enhances endogenous cytokinin levels and inhibits cytokinin oxidase, the enzyme responsible for degrading free adenine-type cytokinins (Guo et al., 2011). Low TDZ concentrations (1.0–2.0 ppm) have proven effective for in vitro propagation of *Rauvolfia tetraphylla* L. (Faisal et al., 2006), sweet potato (Masekesa et al., 2016), and *Phalaenopsis amabilis* (L.) Blume Orchid (Mose dkk., 2017). In *Colocasia esculenta* var. *satoimo*, 0.6 mg L^{-1} TDZ yielded an average of 6.9 shoots per explant (Karyanti & Kartini, 2017), and 0.5 mg L^{-1} TDZ maximized shoot and leaf production in *Vanda douglas* orchids (Karyanti, 2017). In contrast, Nazhira et al., (2024) reported that, in the absence of TDZ, BAP was most effective for shoot multiplication in *Curcuma zeodaria*, whereas Murgayanti et al., (2021) found that TDZ consistently enhanced new shoot formation in the same species. BAP has also been shown to increase shoot numbers in *Kaempferia parviflora*, up to 2.5–3.4 shoots per explant, as reported by Restanto et al., (2024), Zuraida et al., (2015) and Kafindra et al., (2015). However, TDZ outperforms BAP in inducing shoot proliferation (Ramadhani, 2023; Putri, 2016). This superior efficacy of low-concentration TDZ over other cytokinins, such as kinetin and BAP, has been widely documented (Asra et al., 2020; Setiadi et al., 2025).

Number of Leaves

Leaves were counted when fully expanded (i.e., with blades completely unfurled and not curled), including those exhibiting initial chlorosis. Leaf counts were recorded at the end of the eight-week culture period (8 WAC). Treatment P_6 (2 mg L^{-1} TDZ) produced the highest mean number of leaves. As the primary photosynthetic organ, leaf development is strongly influenced by both nutrient availability and TDZ application. Previous studies have demonstrated that exogenous TDZ significantly increases leaf number in various species, as reported by Setiadi et al., (2025); Suminar et al., (2017); Supriati et al.,(2006). The balance between exogenously supplied plant growth regulators (PGRs) and endogenous PGRs within the explant governs leaf initiation (Salifah et al., 2011). In *K. parviflora*, endogenous auxin interacts with media-supplemented TDZ to enhance cell enlargement and division, leading to increased leaf elongation and number, as reported by Suhentaka & Sobir, (2010); Sakina et al., (2019).

CONCLUSION

The present study demonstrated that treatment P_6 (2 mg L^{-1} TDZ) was most effective for both shoot proliferation and leaf production in *Kaempferia parviflora*. TDZ outperformed both BAP and kinetin in inducing shoot multiplication, attributable to its high efficacy even at low concentrations. These findings indicate that in vitro propagation using MS medium supplemented with TDZ can reliably generate the large quantities of high-quality planting material required to support the expanding biopharmaceutical use of black ginger.

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