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Shoot Induction of *Dendrobium lasianthera* J.J.Smith with Several Types of Cytokinins through *In Vitro* Culture

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Abstract: *Dendrobium lasianthera* (Orchidaceae) is one of the potential plants as ornamental plants because it has beautiful and long-lasting flowers. Propagation of orchid plants through *in vitro* culture technique has been widely done to meet market demand. One of the supporting factors in plant propagation through *in vitro* culture techniques is growth regulators. Cytokinin is one of the growth regulators commonly used in plant propagation, including orchids. The study aims to determine the effect of several cytokinins and find the best type for shoot induction of *Dendrobium lasianthera.* The method used a Completely Randomized Design (CRD) with 4 treatments and 6 replications with several types of cytokinis (control; BAP 1.2 mg/L, Kinetin 2 mg/L, and Thidiazuron 1 mg/L) in Murashige & Skoog (MS) media. The results showed that several cytokinins significantly affected the number of shoots, time of shoot appearance, and shoot length of *D. lasianthera.* Thidiazuron was the best cytokinin for *in vitro* shoot induction of *D. lasianthera*.

Keywords: Cytokinin; *Dendrobium lasianthera*; *In Vitro*; Shoot Induction

Introduction

Orchidaceae is the most common group of ornamental plants in the world. *Dendrobium lasianthera* (Orchidaceae) is one type of orchid with beautiful flowers combining red, purple, pink, and white. The demand for this orchid is also increasing along with its diverse use, so its propagation needs to be increased (Utami et al., 2016). Some factors contributing to tissue culture techniques include seed maturity, genotype, media, growth regulators, carbohydrates, and explant type (Tan et al., 2013; Reddy et al., 2021). Cytokinin is one of the growth regulators commonly used in plant propagation, including orchids (Arli et al., 2023). Cytokinin stimulates cell division and shoot growth in tissue culture. Cytokinins have various types, such as BAP, Kinetin, Thidiazuron. BAP is an adenine-derived cytokinin that stimulates plant cell division and shoot multiplication (Azis et al., 2017). Kinetin is a group of cytokinin plant growth regulators that stimulate shoots formation (Lestari, 2013). Thidiazuron is a cytokinin that can induce shoot emergence (Sari et al., 2015).

Various types of cytokinins have been widely used in orchid tissue culture to support the growth of orchid plants. Cytokinins play a role in cell growth, cell differentiation, apical dominance and shoot growth. Cytokinins that are often used in tissue culture are BA, BAP, kinetin, and TDZ. If the availability of cytokinins in medium is limited, cell division will be inhibited, but if medium have adequate cytokinin content, cell division will occur quickly (Markal et al., 2015).

Lo et al. (2022) reported that 1.2 mg L-1 BAP is effective for PLB growth in *Phalaenopsis* orchids. According to Jaiphet & Rangsayaatorn (2010), 2 mg/L kinetin is the optimal concentration for developing PLB from thin protocorms in *Dendrobium gratiosissimum* orchids. Research by Wattanapan et al. (2018) showed that 1 mg L-1 Thidiazuron produced the highest percentage of protocorm regeneration, number of roots, number of shoots, and percentage of live explants in *Paphiopedilum callosum* orchid.

Research regarding the use of cytokinin growth regulators has been commonly carried out. Benzyl Adenine effective for proliferation PLB in *Dendrobium*

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lasianthera orchid (Bawonoadi et al., 2017), BAP effective for PLB growth in *Dendrobium lasianthera* orchid (Mayrendra et al., 2022), and BA effective for induction of somatic embryogenesis in *Dendrobium lasianthera* orchid (Sasmita et al., 2022). This study aimed to determine the effect of several types of cytokinins for shoot induction of *Dendrobiumlasianthera* orchid and find the best type of cytokinins.

Method

Location and Time

The research was conducted from Januari 2023 to April 2023 at Laboratory of Plant Physiology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

Research Design

The research used the experimental method. The research design in this study was completely randomized design (CRD) with 4 treatments and 6 replications with several types of cytokinis (control; BAP 1.2 mg/L, Kinetin 2 mg/L, and Thidiazuron 1 mg/L) in Murashige & Skoog (MS) media.

Shoot Induction

The explants used in this research were *Dendrobium lasianthera* plantlets. Explants were planted in a Laminar Air Flow (LAF) cabinet using a scalpel, tweezers, and a petri dish. Stem of D. lasianthera was cut transversely 1 cm and then planted in the treatment medium. The culture bottle containing the explant is then labeled with a bottle cap.

Research Parameter

Percentage of explant survival (%)

The percentage of explant survival is calculated using the Equation 1:

$$\mathrm{SR}(\%) = \left(\frac{\mathrm{Nt}}{\mathrm{No}}\right) \times 100\% \quad (1)$$

Description:
SR: explant's survival rate;
Nt: number of explants alive on day t;
No: number of explants at the start of maintenance.

Time of Shoot Appearance (Days)

The time of shoot appearance is measured from the beginning of planting until the formation of shoots in days.

Number of Shoots

Number of shoots is measured at the end of the observation (week 8) by counting the number of shoots formed from each explant.

Shoot Length (mm)

Shoot length is measured at the highest shoot using millimeter paper which is measured from the place where the shoot appeared (base) to the tip of the highest shoot. Measurements were carried out 8 weeks after planting.

Data Analysis

The data were analyzed statistically using ANOVA in the SPSS version 20 program. If the effect of the treatment is significantly different, then continue with Duncan's New Multiple Range Test (DNMRT) with a level of 5%.

Result and Discussion

Percentage of Explant Survival (%)

The survival percentage of *Dendrobium lasianthera* explants with the addition of several types of cytokinins is presented in Table 1.

Table 1. The Average percentage of explant survival of *Dendrobium lasianthera* orchids with several types of cytokinins

The Types of Cytokinins	The Average percentage of explant survival (%)
Control	100
BAP 1.2 mg/L	100
Kinetin 2 mg/L	100
Thidiazuron 1 mg/L	100

Adding several types of cytokinins to the MS medium showed that all *D. lasianthera* explants had a survival percentage of 100% (Table 1). All types of cytokinins used in this study had the same effect on the percentage of live explants. These results are align with the research results reported Shen et al. (2018), the survival rate of *Tolumnia* Louise Elmore 'Elsa' after one month of culture was 100%.

Micro-propagated plants have been observed to respond positively to CK treatments, increasing survival rate of explant. This increase is believed to result from higher concentrations of CKs used during micropropagation. Even though the results of the current study have not shown a particularly significant difference among the different CKs, other studies have highlighted the differences that arise from using CKs belonging to different groups (Hlophe et al., 2020).

According to Supriyadi (2014) and Sundari et al. (2015), the percentage of explant survival is influenced by the source of the explant, type, and composition of the media. In this study, node explants of *D. lasianthera* responded well to the percentage of explants survival. Medium MS is quite suitable for *D. lasianthera*.

Time of Shoot Appearance (Days)

The time of shoot appearance of *Dendrobium lasianthera* with the addition of several types of cytokinins is presented in Table 2.

Table 2. The Average time of shoot appearance of *Dendrobium lasianthera* orchids with several types of cytokinins

The Types of Cytokinins	The Average time of shoot appearance (Days)
Control	17.00 c
BAP 1.2 mg/L	14.25 b
Kinetin 2 mg/L	11.00 a
Thidiazuron 1 mg/L	09.00 a

Notes:
Numbers followed by the same lowercase letter are not significantly different in the DNMRT test at the 5% level.

The fastest shoot appearance of *D. lasianthera* was in the TDZ and Kinetin treatments, followed by BAP and control. Thidiazuron 1.0 mg/L and Kinetin 2 mg/L had no significantly different effect on the shoot appearance of *D. lasianthera* (Table 2). 1.0 mg/L Thidiazuron and 2.0 mg/L Kinetin were the best treatment for the shoot appearance of *D. lasianthera*.

TDZ affects plant physiology, such as cell nutrition, and can maintain a balance of endogenous and exogenous hormones (Ouyang et al., 2016; Saifuddin, 2016). In tissue culture systems, it has been proposed that TDZ acts through the adenine-type cytokinin activity either by stimulating endogenous cytokinins or binding to cytokinin receptors (Hothorn et al., 2011; Nisler et al., 2018). Kinetin is a cytokinin hormone group that stimulates shoot formation (Lestari, 2013). According to Restanto et al. (2018), the right concentration and type of cytokinin can stimulate shoot growth.

Number of Shoots

The number of shoots of *D. lasianthera* with the addition of several types of cytokinins is presented in Table 3.

Table 3. The Average number of shoots of *Dendrobium lasianthera* orchids with several types of cytokinins

The Types of Cytokinins	The Average Number of Shoots
Control	1.50 b
BAP 1.2 mg/L	2.50 b
Kinetin 2 mg/L	1.75 b
Thidiazuron 1 mg/L	4.25 a

Notes:
Numbers followed by the same lowercase letter are not significantly different in the DNMRT test at the 5% level.

Thidiazuron 1.0 mg/L had a significantly different effect on the explants than other treatments (Table 3). 1.0 mg/L Thidiazuron was the best treatment for the number of shoots of *D. lasianthera*. Thidiazuron affects the processes of cell division, seed germination, and shoot multiplication (Widyastuti & Deviyanti, 2018). According to Wattanapan et al. (2018), adding 1 mg/L Thidiazuron resulted in the highest percentage of shoots in the *Paphiopedilum callosum* orchid.

Thidiazuron (TDZ) is a urea derivative that acts as a cytokinin-active plant growth regulator in micropropagations to supplement media such as Murashige and Skoog medium. TDZ promotes plant organogenesis (shoot regeneration) and regeneration (Debnath, 2018; Guo et al., 2011; Sujjaritthurakarn & Kanchanapoom, 2013). For example, TDZ induced regeneration in African violets (Saintpaulia ionantha) (Padmanabhan et al., 2014, 2015).

Shoot Length (mm)

Shoot length of *Dendrobium lasianthera* with the addition of several types of cytokinins is presented in Table 4.

Table 4. The Average shoot length of *Dendrobium lasianthera* orchids with several types of cytokinins

The Types of Cytokinins	The Average Shoot Length
Control	10.00 cd
BAP 1.2 mg/L	10.50 bcd
Kinetin 2 mg/L	12.75 b
Thidiazuron 1 mg/L	18.00 a

Notes:
Numbers followed by the same lowercase letter are not significantly different in the DNMRT test at the 5% level.

Thidiazuron 1.0 mg/L significantly affected the average shoot length of the *D. lasianthera* compared to other treatment (Table 4). 1.0 mg/L Thidiazuron was the best treatment for shoot length parameters. The result is align with the research results of Sari et al. (2015). Arafa et al. (2021), reported that 1 mg/L Thidiazuron was effective in shoot formation on *Dendrobium nobile*.

Thidiazuron (TDZ) is plant tissue culture's most active cytokinin substance. It facilitates efficient micropropagation of many recalcitrant species. Low concentrations (< 1 µM) can induce more significant axillary proliferation than other cytokinins; however, TDZ may inhibit shoot elongation. In some cases, it is necessary to transfer shoots to an elongation medium containing a lower level of TDZ and a less active cytokinin. At concentrations higher than 1 µM, TDZ can stimulate the formation of callus, adventitious shoots, or somatic embryos (Huetteman & Preece 2013; Kieber & Schaller 2018; Werner & Schmülling 2009; Zürcher & Müller 2016).

TDZ, characterized as a cytokinin but demonstrating dual auxin and cytokinin-like effects in

diverse plant species when utilized in vitro, presents an intriguing puzzle regarding its mode of action (Miguel et al., 2020). While several hypotheses have been proposed, the precise mechanism remains elusive. One plausible explanation suggests that TDZ might facilitate the accumulation or synthesis of native plant growth hormones, contributing to its observed effects (Maity et al., 2016). The promotion of growth such as callus formation, shoot regeneration, and somatic embryo formation take place when applied at higher concentration and axillary proliferation at comparatively lower concentrations (Haddadi et al., 2013; Ugandhar & Venkateshwarlu, 2015; Afrasiab et al., 2017). It might be due to its similar biological activity like other N6-substituted cytokinins such as N-N-diphenylurea and N-(2-chloro-4-pyridyl)-N′-phenylurea.

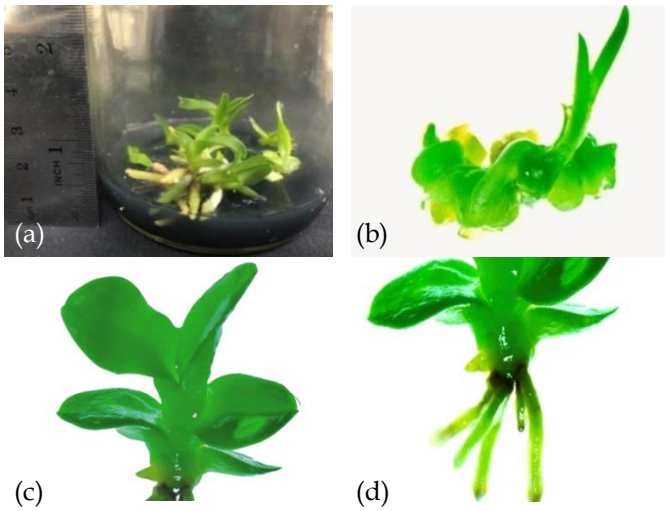

Figure 1. Response of *Dendrobium lasianthera* orchid with several types of cytokinins. (a) planlet; (b) shoots; (c) leaves; (d) roots.

Conclusion

Types of cytokinins significantly affected the number of shoots, time of shoot appearance, and shoot length of *D.lasianthera*. Thidiazuron was the best cytokinin for *in vitro* shoot induction of *D. lasianthera*.

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Author Contributions
Conceptualization, N.M.A. and Z.A.N.; methodology, Z.A.N.; software, N.M.A.; validation, N.M.A. and Z.A.N.; formal analysis, N.M.A.; investigation, N.M.A.; resources, N.M.A.; data curation, N.M.A.; writing—original draft preparation, N.M.A.; writing—review and editing, Z.A.N.; visualization, N.M.A.; supervision, Z.A.N.; project administration, N.M.A. and Z.A.N.

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Conflicts of Interest
The authors declare no conflict of interest.

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