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Research Article

Effect of Benzyl-Adenine and Thidiazuron on *In Vitro* Multiplication of Ginger (*Zingiber officinale* Rosc.) Shoots

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ABSTRACT

The wilt disease caused by *Ralstonia solanacearum* and the leaf spot disease caused by *Phyllosticta* sp. are significant co*n*straints in ginger cultivation as they can lead to crop failure. One approach to eliminating these diseases is to use disease-free ginger plantlets obtained through tissue culture propagation. This study investigated the influence of plant growth regulators, i.e., Benzyl Adenine (BA) and Thidiazuron (TDZ), on the in vitro multiplication of large white ginger shoots. The tested treatments included combinations of BA (0, 1, 2, 3 mg L^{-1}) and TDZ (0, 0.1, and 0.2 mg L^{-1}), with ten replicates each. A complete randomised factorial experimental design was employed. The observed variables were shoot height, number of shoots, number of leaves, and number and length of roots at 2, 4, 6, and 8 weeks of age. The results indicated an interaction between TDZ and BA for shoot number and root length. The highest numbers of shoots were obtained after eight weeks using 0.1 mg L^{-1} TDZ alone without BA. Meanwhile, the longest roots were obtained after eight weeks using a specific combination of TDZ and BA concentrations. Based on this study, we proposed a strategy to implement this protocol to induce the formation of shoots, leaves, and roots in a multistep tissue culture propagation.

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INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is an important medicinal plant belonging to the *Zingiberaceae* family (Shahrajabian et al. 2019a). In Indonesia, there are three types of ginger known based on size, shape, and color: large white ginger, red ginger, and small white ginger (also known as *emprit* ginger) (Sabulal et al. 2006; Guji et al. 2019). Ginger has been widely used in traditional medicine and is now utilised for producing herbal medicine standards (OHT) and phytopharmaceuticals (Sabulal et al. 2006; Azhari et al. 2017; Sharifi-Rad et al. 2017; Shahrajabian et al. 2019b). Large white ginger is primarily used as a culinary spice, while red ginger is more commonly used in traditional medicine (Shahrajabian et al. 2019a). Ginger rhizome is also widely used as a spice or condiment (El Sayed et al. 2016).

Ginger contains a diverse range of bioactive compounds, such as gingerol, shogaol, and zingerone (Sharifi-Rad et al. 2017; Shahrajabian et al. 2019b; Shahzad et al. 2023). Ginger has various nutritional and medicinal importance (Shahzad et al. 2023). These compounds have been shown to exert various pharmacological activities, including antioxidant, anti-inflammatory, analgesic, and anti-carcinogenic effects (Sharifi-Rad et al. 2017). For instance, gingerol and shogaol have antioxidant activities scavenging reactive oxygen species (Sharifi-Rad et al. 2017). Ginger extracts have exhibited anti-inflammatory effects by inhibiting pro-inflammatory cytokines (Sharifi-Rad et al. 2017). The analgesic activity of ginger may be attributed to modulation of neurotransmitters and suppression of prostaglandin synthesis. Gingerols play an important role in the alleviation of arthritis and pain (Shahzad et al. 2023). Further research into the pharmacological mechanisms and clinical efficacy of these ginger compounds is warranted. Overall, the presence of these bioactives contributes to the wide use of ginger as both a versatile culinary ingredient and medicinal plant (Semwal et al. 2015).

However, bacterial wilt caused by *Ralstonia solanacearum* and leaf spot caused by *Phyllosticta* sp. are major diseases that constrain ginger cultivation (Chaidir et al. 2019). These diseases pose a significant threat, as severe infestations can result in plant death and crop failure (Sitinjak 2010; Adriani et al. 2012). Both plant pathogens affect all types of ginger, including large white ginger, red ginger, and small white ginger, decreasing national ginger productivity (Fauzia & Nurcahyanti 2020).

One approach to mitigating these diseases in ginger is to use healthy ginger seeds from tissue culture propagation. This technology offers the advantage of obtaining disease-free seedlings (Kumar & Reddy 2011; Seran 2013; Lestari et al. 2023). Tissue culture propagation using disease-free ginger plantlets is one potentially effective way to obtain healthy planting material that can help ginger farmers combat disease pressures and improve productivity, offering increased yields, better quality, cost savings, and new market opportunities (Azhari et al. 2017; Sharifi-Rad et al. 2017; Shahrajabian et al. 2019a, 2019b).

Tissue culture technology has been widely applied to support agricultural development, including mass seed production, development of superior varieties, germplasm preservation, and secondary metabolite production (Kumar & Reddy 2011; Lestari 2016; Tegen & Mohammed 2016). The success of plant propagation through tissue culture is influenced by various factors, including the growth media composition, the source of explants, and the types of plant growth regulators (PGRs) used (Gupta et al. 2020; Zahid et al. 2021). Among the PGRs, cytokinin, particularly benzyl-adenine (BA), is commonly used to stimulate shoot proliferation (Lestari 2011; Mehaboob et al. 2019). BA is a cytokinin with strong activity compared to kinetin (Lestari 2011; Grąbkowska et al. 2014).

The aim of this study was to investigate the effects of different concen-

trations of benzyl-adenine (BA) and thidiazuron (TDZ) on *in vitro* propagation of ginger.

MATERIALS AND METHODS

Materials

The plant material used as explants was shoot buds obtained from rhizomes of the 'Cimanggu 1' variety of large white ginger (*Zingiber officinale*). This variety is susceptible to *Ralstonia solanacearum* (Ministry of Agriculture 2001). This ginger variety was obtained from a farmer's plantation in Sumedang, West Java, Indonesia. The collected ginger rhizomes were maintained in a greenhouse to provide a ready source of explants for experiments.

Methods

The research was conducted from March to December 2021 at the Tissue Culture Laboratory, Indonesia Centre for Agricultural Biotechnology and Genetic Resources (ICABIOGRAD), Cimanggu 3, Bogor, West Java, Indonesia. The collected ginger rhizomes were washed thoroughly with soap and water to remove debris. The cleaned rhizomes were then placed in 20 cm x 40 cm plastic containers and incubated in darkness for 48 hours. Before culturing in the medium, the explants were sterilised by soaking and rubbing them in 70 % alcohol for 5 minutes, followed by 20 % Clorox treatment for 10 minutes, and finally rinsing them three times with sterile distilled water. The sterilised explants were then planted on MS basal medium (Murashige & Skoog 1962) without plant growth regulators (PGRs) to obtain sterile shoot buds. To initiate and proliferate new axillary shoots from the rhizome explants, rhizome segments were cultured in vitro on Murashige and Skoog medium supplemented with 1.0 mg L^{-1} of the benzyl-adenine (BA) under low light for 4 weeks.

The basal medium used consisted of MS composition supplemented with 30 mg L^{-1} sucrose, 100 mg L^{-1} myoinositol + vitamin B group (thiamin, glycine, pyridoxine, and nicotinic acid), and 2 mg L^{-1} of gelrite as a gelling agent. The pH of the medium was adjusted to 5.8 using 1 N NaOH or HCl during pH measurement.

The media were sterilised using an autoclave at 121 °C. The explants were planted in laminar flow cabinets, with one bottle containing one explant of one cm-sized shoot bud. The plant explants were cultured in glass bottles that were arranged on racks inside a growth room dedicated for tissue culture. The room was equipped with neon lamps connected to an automated timer system, which provided 16 hours of light exposure per day at an intensity of 1500 lux. The room temperature ranged from 21 °C to 23 °C, with around 90 % humidity.

Shoots that resulted from the initial stage were subcultured on MS medium supplemented with 1.0 mg L^{-1} BA to obtain sufficient shoots for the treatment. The test media compositions were BA (0, 1, 2, 3 and 5 mg L^{-1}) and TDZ (0, 0.1, and 0.2 mg L^{-1}). Each treatment consisted of 10 bottles, resulting in a total of 120 bottles with 14 treatments, including BA (four concentrations) and TDZ (three concentrations).

Data Analysis

A completely randomised design with a factorial pattern was used for the experimental design. The variables observed were shoot height measured from the base to the tip of the shoot (cm), number of shoots, number of leaves, and number and length of roots at 2, 4, 6, and 8 weeks after planting (WAP). The data were analysed using SAS software. An analysis of variance was performed to determine the best treatment, and regression testing was used to determine the optimum dosage of PGRs.

RESULTS AND DISCUSSION

Influence of TDZ and BA treatment upon the height of ginger shoots

The analysis of variance for the shoot height variable indicated no interaction between TDZ and BA (Table 1). The use of 0.0 mg L^{-1} TDZ and 0.0 mg L^{-1} BA showed the highest shoots on week 8 (Tables 2 and 3).

Shoot elongation of ginger was faster in explants treated by the addition of TDZ compared to BA, indicated by the differences in the average height of ginger shoot during the 8-week culture duration. TDZ at 0.2 mg L^{-1} resulted in a significantly taller shoot versus other treatments from 2 to 8 weeks. Shoots treated with 0.2 mg L^{-1} TDZ were approximately 2–3 times longer than shoot treated with BA alone. The superior effect of TDZ on shoot height stimulation was likely due to its higher cytokinin activity, which promoted cell division and elongation. In contrast, BA had a weaker influence on these growth parameters. Overall, TDZ was more effective than BA for enhancing shoot elongation of ginger *in vitro*.

Table 1. Result of the analysis of variance for the height of ginger shoots.

Week After Planting (WAP)	Middle Square				CV (%)
	Replication	TDZ	BA	TDZ*BA	
2	0.201*ns*	1.276**	0.529*	0.172*ns*	28.82
4	0.506*	5.027**	0.358*ns*	0.222*ns*	28.57
6	0.288*ns*	10.005**	1.034*ns*	0.310*ns*	31.15
8	0.483*ns*	15.845**	11.925**	0.487*ns*	35.54

Note: statistical significance based on F-test $\alpha = 0.05$ - *ns* is not significantly different; (*) significantly different; (**) highly significantly different; CV = coefficient of variation. BA = Benzyl-adenine. TDZ = Thidiazuron. WAP = Weeks After Planting.

Table 2. The influence of TDZ on the height of ginger shoots.

Concentration of TDZ (mg L^{-1})	Average Shoot Height (cm)			
	2 WAP	4 WAP	6 WAP	8 WAP
0	1.47a	2.01a	2.57a	3.59a
0.1	1.20b	1.41b	1.70b	2.52b
0.2	1.14b	1.38b	1.71b	2.42b
Mean	1.27	1.6	1.99	2.85
F-Value of TDZ	9.58**	24.03**	25.97**	16.22**

Note: statistical analysis result based on Duncan's post-hoc test, number followed by the same letter in the same column considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Table 3. The influence of BA on the height of ginger shoots.

Concentration of BA (mg L^{-1})	Average Shoot Height			
	2 WAP	4 WAP	6 WAP	8 WAP
0	1.12c	1.63	2.03	3.62a
1	1.20bc	1.73	2.23	2.98b
3	1.42a	1.57	1.9	2.67b
5	1.32ab	1.47	1.81	2.07c
Mean	1.27	1.6	1.99	2.83
F-Value of BA	3.97*	1.71*ns*	2.68*ns*	11.71**

Note: statistical analysis result based on Duncan's post-hoc test, number followed by the same letter in the same column considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Influence of TDZ and BA treatment upon the number of ginger shoots

Analysis of variance showed a significant interaction between TDZ and BA on the number of ginger shoots (Table 4). Further analysis of this interaction at 8 weeks revealed the combination of 0.1 mg L^{-1} TDZ with 0 mg L^{-1} BA gave the highest shoots multiplication rate, resulting in an average of 6.3 shoots per explant (Figure 1). There was no significant difference in shoot number between 0.1 mg L^{-1} TDZ + 1.0 mg L^{-1} BA, 0.2 mg L^{-1} TDZ + 1.0 mg L^{-1} BA, and 0.2 TDZ + 0 mg L^{-1} BA after 8 weeks (Table 5).

Using TDZ at 0.1 mg L^{-1} without any BA supplementation gave the highest number of ginger shoots after 8 weeks. This indicates that 0.1 mg L^{-1} TDZ was optimal for shoot multiplication. Thidiazuron (TDZ) is a plant growth regulator that has shown both auxin and cytokinin-like effects despite chemically different from commonly used auxins and cytokinins. TDZ has been found to induce a wide array of physiological and biochemical events in cells (Zahid et al. 2021). At low concentrations (0.1-0.5 mg L^{-1}), TDZ plays a role in stimulating cell division and can be used alone or in combination with cytokinin (Gupta et al. 2020). TDZ has been utilised to promote shoot multiplication in several perennial plants (Mehaboob 2019).

Table 4. Result on the analysis of variance for the number of ginger shoots.

Week of observation	Middle Square				CV (%)
	Replication	TDZ	BA	TDZ*BA	
2	0.411*ns*	0.775*ns*	1.511**	0.519*ns*	42.95
4	2.352*ns*	19.658**	10.764**	4.481*	53.65
6	3.689*ns*	18.808**	18.355**	6.864*	58.56
8	2.87*ns*	19.560*	37.705**	11.657*	55.47

Note: statistical significance based on F-test $\alpha = 0.05$ - *ns* is not significantly different; (*) significantly different; (**) highly significantly different; CV = coefficient of variation. BA = Benzyl-adenine. TDZ = Thidiazuron. WAP = Weeks After Planting.

Table 5. Interaction of TDZ and BA upon addition to the number of ginger shoots.

Concentration of TDZ (mg L^{-1})	Concentration of BA (mg L^{-1})			
	0	1	3	5
	Average Number of Shoots (4 WAP)			
0	1.40f	1.80def	1.50f	2.00cdef
0.1	2.90bcde	3.80ab	1.60ef	2.10cdef
0.2	3.20abc	4.20a	3.00abcd	1.80def
	Average Number of Shoots (6 WAP)			
0	1.60d	2.50bcd	2.10cd	2.50bcd
0.1	4.10ab	4.60a	1.90d	2.90bcd
0.2	3.70abc	4.90a	2.80bcd	2.00cd
	Average Number of Shoots (8 WAP)			
0	2.50e	3.90bcde	2.90e	3.00de
0.1	6.30a	5.90ab	2.40e	3.20cde
0.2	5.20abc	5.10abcd	3.70cde	2.20e

Note: statistical analysis result based on Duncan's post-hoc test, number followed by the same letter in the same column considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Figure 1. In vitro culture of *Zingiber officinale* Rosc. Cimanggu 1 Variety.

Lestari (2011) reported that a combination treatment of BA with TDZ increased the number of shoots compared to treatments without PGRs in cassava propagation in vitro. The proliferation of *Harpagophytum procumbens* plants (Grąbkowska et al. 2014) showed similar results: soaking using 25 mol L^{-1} TDZ for 6 hours produced more vigorous shoots when the plantlets were acclimatised. The cultures originating from TDZ treatments exhibited better growth in shoot length, leaf size, flowering phase, and faster root formation (Tegen & Mohammed 2016). Another study by Grąbkowska et al. (2014) revealed that *in vivo* shoot multiplication rates and sucker growth of banana cv. Mzuzu, Bukoba, and Mtwike can be increased by dipping de-sheathed corms in a TDZ solution at 2.0 mg L^{-1} for 12 hours. TDZ was also more effective than BA on shoot proliferation (Shaheen 2020).

Influence of TDZ and BA treatment on the number of Ginger Leaves

The analysis of variance for the number of leaves variable indicated no interaction between TDZ and BA (Table 6). The use of thidiazuron (TDZ) showed significantly different results on week 4, while BA treatment showed significant differences on weeks 2, 4, 6, and 8 (Tables 7 and 8).

Influence of TDZ and BA treatment on the number of ginger roots

The analysis of variance for the number of root variables indicated no interaction between the use of TDZ and BA (Table 9). The application of TDZ significantly affected the number of roots at 2, 4, and 6 weeks after planting (WAP), while the BA treatment significantly affected the respo*n*se of the number of roots at 6 and 8 WAP (Table 10, 11).

The treatment without BA resulted in a response in the number of roots that was not significantly different from BA concentrations of 1.0 mg L^{-1} and 3.0 mg L^{-1} at 6 WAP and 8 WAP. It was not significantly different from BA 1.0 mg L^{-1} and significantly higher than BA concentrations of 3.0 mg L^{-1} and 5 mg L^{-1}. For the variable of the number of roots, the application of BA was more effective at low concentrations (1-3 mg L^{-1}). Increasing the concentration up to 5 mg L^{-1} tended to decrease the number of roots. In this experiment, rooting in vitro revealed the greatest roots by application of MS + BA 0 mg L^{-1} (13.07 roots). The addition of BA and TDZ to the medium had no significant effect on the number of roots produced. The cultural response to the addition of in vitro growth regulators is different for each plant including ginger.

Table 6. Result of the analysis of variance of the number of ginger leaves.

Week After Planting (WAP)	Middle Square				CV (%)
	Replication	TDZ	BA	TDZ*BA	
2	0.348*ns*	1.225*ns*	2.156*	1.114*ns*	75.38
4	1.297*ns*	12.700*	10.719*	3.878*ns*	55.24
6	12.018*ns*	15.808*ns*	51.178*	16.419*ns*	61.6
8	28.045*ns*	8.081*ns*	311.386**	49.995*ns*	58.56

Note: The statistical significance based on F-test $\alpha = 0.05$ - *ns* was not significantly different; (*) significantly different; (**) highly significantly different; CV = coefficient of variation. BA = Benzyl-adenine. TDZ = Thidiazuron. WAP = Weeks After Planting.

Table 7. The effect of TDZ concentration on the number of ginger leaves.

Concentration of TDZ (mg L^{-1})	Number of Leaf Count			
	2 WAP	4 WAP	6 WAP	8 WAP
0	1.3	2.68b	5.63	10.62
0.1	1.03	2.73b	4.45	9.75
0.2	0.98	3.68a	5.43	9.8
Mean	1.1	3.03	5.17	10.06
F-Value of TDZ	1.78*ns*	4.55*	1.56*ns*	0.23*ns*

Note: The statistical analysis result was based on Duncan's post-hoc test, and the number followed by the same letter in the same column was considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Table 8. The influence of BA concentration on numbers of ginger leaves.

Concentration of BA (mg L^{-1})	Numbers of Leaves			
	2 WAP	4 WAP	6 WAP	8 WAP
0	0.97b	3.43a	6.43a	12.93a
1	1.50a	3.57a	6.10a	12.57a
3	1.00b	2.83ab	4.40b	8.37b
5	0.93b	2.27b	3.73b	6.21b
Mean	1.1	3.03	5.17	10.02
F-Value of BA	3.14*	3.84*	5.05**	8.99**

Note: The statistical analysis result was based on Duncan's post-hoc test, and the number followed by the same letter in the same column was considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Table 9. Analysis of variance for the number of ginger roots.

Week After Planting (WAP)	Middle Square				CV (%)
	Replication	TDZ	BA	TDZ*BA	
2	1.752**	2.844*	0.882*ns*	0.830*ns*	81.33
4	2.160*ns*	46.087**	3.903*ns*	1.870*ns*	68.34
6	8.218*ns*	187.534**	34.932*	5.744*ns*	72.22
8	22.327*ns*	105.125*ns*	305.189**	32.850*ns*	62.92

Note: statistical significance based on F-test $\alpha = 0.05$ - *ns* was not significantly different; (*) significantly different; (**) highly significantly different; CV = coefficient of variation. BA = Benzyl-adenine. TDZ = Thidiazuron. WAP = Weeks After Planting.

Table 10. Effect of TDZ on the number of ginger roots.

Concentration of TDZ (g L^{-1})	Number of Roots			
	2 WAP	4 WAP	6 WAP	8 WAP
0	1.30a	3.43a	6.83a	11.48
0.1	0.95ab	1.82b	3.33b	9.7
0.2	0.78b	1.38b	2.85b	8.26
Mean	1.01	2.21	4.33	9.81
F-Value of TDZ	4.23*	19.98**	18.72**	2.74ns

Note: The statistical analysis result was based on Duncan's post-hoc test, and the number followed by the same letter in the same column was considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Table 11. Effect of BA on the number of ginger roots.

Concentration of BA (g L^{-1})	Number of Roots			
	2 WAP	4 WAP	6 WAP	8 WAP
0	1.07	2.38	5.07a	13.07a
1	1.17	2.53	5.27a	11.41ab
3	1.03	2.29	4.30ab	9.04b
5	0.77	1.7	2.86b	5.69c
Mean	1.01	2.22	4.37	9.8
F-Value of BA	1.31ns	1.69ns	3.49*	7.96**

Note: statistical analysis result based on Duncan's post-hoc test, number followed by the same letter in the same column considered not significant. (*) significantly different at $\alpha = 0.05$; (**) highly significant at $\alpha = 0.01$. WAP = Weeks After Planting.

Influence of TDZ and BA treatment on the length of ginger roots

The analysis of variance for the root length variable indicated an interaction between the use of TDZ and BA at 4 and 8 weeks of age (Table 12). TDZ significantly influenced the response of ginger root length at 2, 4, 6, and 8 WAP, while the BA treatment significantly affected the response of root length at 4, 6, and 8 WAP (Table 13,14).

Table 13 shows that for the variable root length at 4 and 8 weeks after planting, the treatment without TDZ and BA gave responses that were not significantly different from the treatment with TDZ and BA at higher concentrations.

This is an in-depth look at the data on the influence of TDZ and BA on the height of ginger shoots. The highest growth was achieved after 8 weeks. The addition of TDZ (0.1 and 0.2 mg L^{-1}) and BA (1, 3, and 5 mg L^{-1}) resulted in lower height of ginger shoots compared to the results without TDZ and BA supplementation. Based on this result, neither more TDZ nor BA is required to induce ginger shoots' length in vitro.

Based on the results, the highest number of ginger shoots was achieved after week 8 using a combination of TDZ (0.1 mg L^{-1}) and BA (0.0 mg L^{-1}). However, the increase in the number of shoots was an average of one shoot compared to the medium without TDZ (0 mg L^{-1}) and less than one shoot compared to the media without BA (0 mg L^{-1}). The highest number of leaves and the number and length of ginger roots were also achieved without TDZ or BA, as was the highest number and length of ginger roots.

PGR treatments for the ginger rhizome, explant initial propagation, and explant subculture by adding 1 mg L^{-1} BA are considered sufficient for the optimal propagation of ginger. Therefore, there was no additional requirement for supplementation of TDZ and BA afterward. Endogenous phytohormones play important roles in ginger shoot formation and elongation (Lestari 2011). The extra addition of TDZ and BA probably activates negative feedback, thus inhibiting shoots and root formation and elongation instead.

Table 12. Analysis of variance for the variable of root length.

Root length (WAP)	Middle Square				CV(%)
	Replication	TDZ	BA	TDZ*BA	
2	0.356*ns*	3.290**	0.387*ns*	0.619*ns*	88.55
4	1.745**	13.751**	3.952**	1.576*	53.51
6	1.539*ns*	22.131**	9.925**	1.475*ns*	50.44
8	1.015*ns*	14.405**	20.371**	2.061*	33.92

Note: statistical significance based on F-test α = 0.05 - ns was not significantly different; (*) significantly different; (**) highly significantly different; CV = coefficient of variation. BA = Benzyl-adenine. TDZ = Thidiazuron. WAP = Weeks After Planting.

Table 13. Effect of interaction between TDZ concentration and BA on root length

THO Concentration (g L^{-1})	BA Concentration (g L^{-1})			
	0.0	1.0	3.0	5.0
	Root length 4 WAP (cm)			
0.0	1.95abc	2.17ab	2.52a	1.98abc
0.1	1.90abc	1.43bcd	1.03def	0.57ef
0.2	1.21cde	1.73abcd	1.01def	0.34f
	Root length 8 WAP (cm)			
0.0	3.87a	3.64ab	3.68ab	2.79bcd
0.1	3.62ab	3.10abc	2.48cd	1.52ef
0.2	3.03abc	3.48ab	1.91de	0.80f

Note: Data followed by the same letter on the same observation variable is not significantly different based on further tests Duncan α = 0.05.

CONCLUSIONS

The application of 0.1 mg L^{-1} TDZ achieved the highest number of shoots (6 shoots per-explants). The highest number of leaves and shoots height was also achieved without TDZ or BA, as well as the number and length of ginger roots. Efficient propagation requires no additional TDZ and BA afterward to achieve optimal height, number of shoots, number of leaves, and root number and length. Increasing the concentration of BA led to a decrease in the number of shoots and root length. Furthermore, there was no interaction between TDZ and BA treatments concerning plant height or the number of leaves.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of the initial draft of the manuscript, as well as the conception and design of the study, material processing, and data collection. All authors reviewed and approved the final submitted version of the manuscript.

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CONFLICT OF INTEREST

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REFERENCES

Adriani, A.R., Gusnawati, H.S. & Khaeruni, A., 2012. Respon ketahanan berbagai varietas tomat terhadap penyakit layu bakteri (*Ralstonia solanacearum*). *Jurnal Agroteknologi*, 2(2), pp.63-68.

Azhari, H.N., Yap, S.S. & Nour, A.H., 2017. Extraction and chemical compositions of ginger (*Zingiber officinale* Roscoe) essential oils as cockroaches repellent. *Australian Journal of Basic and Applied Sciences*, 11 (3), pp.1-8.

Chaidir, L., 2019. Effect of sucrose on in vitro bud multiplication of torch ginger (*Etlingera elatior*). *IOP Conference Series: Earth and Environmental Science*, 334(1), pp.1-3. doi: 10.1088/1755-1315/334/1/012015.

El Sayed, S.M. & Moustafa, R. A., 2016. Effect of combined administration of ginger and cinnamon on high-fat diet induced hyperlipidemia in rats. *Journal of Pharmaceutical, Chemical and Biological Sciences*, 3(4), pp.561-572.

Fauzia, Y.F. & Nurcahyanti, S.D., 2020. Ketahanan tiga klon jahe (*Zingiber officinale* Rosc.) terhadap penyakit layu bakteri (*Ralstonia solanacearum*). *Jurnal Proteksi Tanaman Tropis*, 1(2), pp.62-69.

Grąbkowska, R., Sitarek, P. & Wysokińska, H., 2014. Influence of thidiazuron (TDZ) pretreatment of shoot tips on shoot multiplication and ex vitro acclimatization of *Harpagophytum procumbens*. *Acta Physiologiae Plantarum*, 36(7), pp.1661–1672. doi: 10.1007/s11738-014-1541-9.

Guji, M.J., Yetayew, H.T. & Kidanu, E.D., 2019. Yield loss of ginger (*Zingiber officinale*) due to bacterial wilt (*Ralstonia solanacearum*) in different wilt management systems in Ethiopia. *Agriculture & Food Security*, 8, 5. doi: 10.1186/s40066-018-0245-6

Guo, B. et al., 2011. Thidiazuron: A multi-dimensional plant growth regulator. *African Journal of Biotechnology*, 10(45), pp.8984–9000. doi: 10.5897/AJB11.636.

Gupta, N. et al., 2020. A review on micropropagation culture method. *Asian Journal of Pharmaceutical Research and Development*, 8(1), pp.86–93. doi: 10.22270/ajprd.v8i1.653.

Kumar, N. & Reddy, M.P., 2011. *In vitro* plant propagation: a review. *Journal of Forest and Environmental Science*, 27(2), pp.61-72.

Lestari, E.G., 2011. Peranan zat pengatur tumbuh dalam perbanyakan tanaman melalui kultur jaringan. *Jurnal Agrobiogen*, 7(1), pp.63–68.

Lestari, E.G., 2016. *Pemuliaan tanaman melalui induksi mutasi dan kultur in vitro*, First Ed., Indonesia: IAARD Press.

Lestari, E.G., Nugraha, M.F.I. & Yunita, R., 2023. The Formula media *in vitro* Propagation and Conservation of *Ludwigia* sp. *Journal of Tropical Biodiversity and Biotechnology*, 8(1), jtbb75947. doi: 10.22146/jtbb.75947

Mehaboob, V.M. et al., 2019. Direct organogenesis and microrhizome production in ginger (*Zingiber officinale* Rosc.). *Journal of Pharmacognosy and Phytochemistry*, 8(3), pp.2880-2883.

Ministry of Agriculture, 2001. Decree on the release of large white ginger varieties Cimanggu 1, No. 109/Kpts/Tp. 240/2/2001.

Murashige, T. & Skoog, F., 1962. A Revised Medium For Rapid Growth And Bioassay With Tissue Cultures. *Physiologia Plantarum*, 15(3), pp.473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x

Sabulal, B. et al., 2006. Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: Chemical characterization and antimicrobial activity. *Phytochemistry*, 67(22), pp.2469-2473. doi: 10.1016/j.phytochem.2006.08.003

Semwal, R.B. et al., 2015. Important nutraceutical principles from ginger. *Phytochemistry*, 17, pp.554-568. doi: 10.1016/j.phytochem.2015.07.012.

Seran, T.H., 2013. *In vitro* propagation of ginger (*Zingiber officinale* Rosc.) through direct organogenesis: A review. *Pakistan Journal of Biological Sciences*, 16(24), pp.1826–1835. doi: 10.3923/pjbs.2013.1826.1835.

Shahrajabian, M. H., Sun, W. & Cheng, Q., 2019a. Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine and modern industry. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 69(6), pp.546–556. doi: 10.1080/09064710.2019.1606930.

Shahrajabian, M. H., Sun, W., & Cheng, Q., 2019b. Pharmacological uses and health benefits of ginger (*Zingiber officinale*) in traditional asian and ancient chinese medicine, and modern practice. *Notulae Scientia Biologicae*, 11(3), pp. 309–319. doi: 10.15835/nsb11310419.

Shahzad, H. et al., 2023. Medicinal biospecificity of ginger and its efficacious bioactive compounds in the context of its biological activities against predominant health issues: Current study and new avenues. *Biology, Medicine, & Natural Product Chemistry*, 12(1), pp.371–389. doi: 10.14421/biomedich.2023.121.371-389

Shan, X., Li, D., & Qu, R., 2000. Thidiazuron promotes in vitro regeneration of wheat and barley. *In Vitro Cellular & Developmental Biology-Plant*, 36, pp.207-210.

Shaheen A., et al., 2020. Micropropagation of licorice (*Glycyrrhiza glabra* L.) by using intermediate nodal explants. *Chil. J. Agric. Res.*, 80(3), pp.326–333. doi: 10.4067/S0718-58392020000300326.

Sharifi-Rad, M. et al, 2017. Plants of the genus *Zingiber* as a source of bioactive phytochemicals: From tradition to pharmacy. *Molecules*, 22 (12), pp.2-20. doi: 10.3390/molecules22122145.

Sitinjak, R.R., 2010. Pemanfaatan meristem dalam teknik kultur jaringan. *Jurnal Akademia*, 14(4), pp.56–59.

Tegen, H., & Mohammed, W., 2016. The role of plant tissue culture to supply disease free planting materials of major horticultural crops in Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 6(1), pp.122-129.

Zahid, N.A., Jaafar, H.Z.E. & Hakiman, M., 2021. Micropropagation of ginger (*Zingiber officinale* Roscoe) 'bentong' and evaluation of its secondary metabolites and antioxidant activities compared with the conventionally propagated plant. *Plants*, 10(4), 630. doi: 10.3390/plants10040630