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Thidiazuron Improved *Aglaonema* 'Ruby' Microshoot Multiplication for Mass Production and Microfloriculture Development

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Abstract. *Aglaonema 'Ruby' is a hybrid Aglaonema with a dominant green leaf pattern and a red accent in the middle. This cultivar is widely cultivated and in great demand, and it can potentially be used in micro floriculture development. Conventional Aglaonema propagation through stem cuttings can only produce 1-3 shoots. Therefore, the in vitro culture method is proposed. The objectives of this study were to evaluate the effect of three synthetic cytokinins (BAP, Kinetin, and TDZ—the latter being a phenylurea derivative with cytokinin-like activity) and concentrations on the multiplication of Aglaonema 'Ruby' microshoot. The research has been carried out experimentally using a split-plot design. The main plot was cytokinin types, consisting of BAP, Kinetin, and Thidiazuron; the subplot was cytokinin concentrations at 0, 5, 10, 15, and 20 µM. The measured parameters include shoot emergence time, number of shoots, leaves, and shoot length. The data were analyzed using an analysis of variance (ANOVA) followed by Duncan's multiple range test at 95% confidence level. It can be concluded that the growth of Aglaonema 'Ruby' micro shoots was controlled by the type and concentration of cytokinin given. Thidiazuron was better than Kinetin and BAP in stimulating the growth of Aglaonema 'Ruby' microshoots. Cytokinin at 10 µM seemed to be effective in improving Aglaonema 'Ruby' micro shoots multiplication. Thidiazuron at 10 µM can increase the production of Aglaonema 'Ruby' shoot to support both mass production of seedlings and microfloriculture products. Further studies are needed to optimize shoot and root development to produce good plantlets, easing the subsequent acclimatization.*

Keywords: *Aglaonema 'Ruby', BAP, kinetin, microshoot, thidiazuron*

Citation
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INTRODUCTION

Aglaonema, also known as Chinese evergreens, is a monocot plant from the *Araceae* family. *Aglaonema* consists of 21 species distributed in tropical rainforests in Asia (Dikaryani et al., 2019). *Aglaonema* is an ornamental plant with the main attraction on its leaves. *Aglaonema* 'Ruby' is a hybrid *Aglaonema* that has a dominant green leaf pattern with a red accent in the middle and is widely cultivated (Putri et al., 2022); popular, its demand has increased significantly (Wahyuni et al., 2014). The high market demand for *Aglaonema* in Indonesia is not followed by an increase in domestic production, which has led to a monthly import of 600,000 plants (Saptowalyono, 2022). The need for *Aglaonema* exports has also increased by 61%, from 856,521 plants in 2020 to 1,382,243 plants in 2021 (BPS, 2021).

The attractiveness of *Aglaonema* 'Ruby' has great potential for developing microfloriculture, a creative economy product. Microfloriculture is a registered trademark of souvenirs and Living Room Ornaments (LivROnt) based on the in vitro culture of ornamental plants (Restianto et al., 2024). Micro floriculture products are (1) novel and distinctive, (2) eco-friendly, (3) can be mass-produced all year round, and (4) reasonably priced compared to conventional souvenirs (Suliyanto et al., 2022).

Aglaonema propagation is usually done vegetatively through stem cuttings, but the number of shoots produced is limited (1-3 cutting per plant). *Aglaonema* propagation using in vitro culture techniques is offered to meet the increasing demand for *Aglaonema*. Large quantities of seedings can be produced relatively quickly, with the same characteristics as the parental plant, and are disease-free (Phillips & Garda, 2019). The success of plant in vitro culture is controlled by several factors, including the media and growth regulators. One group of growth regulators commonly used for plant propagation is cytokinin. Cytokinins stimulate cell division and the growth of axillary shoots by reducing apical dominance (Kieber & Schaller, 2018).

Cytokinins frequently used in plant in vitro cultures include 6-Benzylaminopurine (BAP), Kinetin, and Thidiazuron (TDZ). According to Zhao et al. (2024), BAP is a synthetic cytokinin that is stable, relatively cheaper, and more effective in inducing lateral shoots. Meanwhile, Kinetin is another cytokinin capable of stimulating cell division in plant in vitro culture (de Oliveira et al., 2022). Li et al. (2021) added that Kinetin can increase transcription and translation activity, accelerating the transition from G2 to M phase in the cell cycle, leading to accelerated cell division and shoot induction. Moreover, TDZ is a phenylurea derivative with cytokinin-like activity, which is very good in inducing organogenesis, regeneration, and development of axillary shoots (Dewir et al., 2018). Brunoni et al. (2021) and Ricci & Rolli (2020) added that TDZ is often used to stimulate initial shoot growth in new cultures. TDZ can increase the action of other exogenous and endogenous cytokinins (Fathy et al., 2022; Ram et al., 2022) and is also capable of stimulating the production of other endogenous cytokinins (Dewir et al., 2018) by inhibiting cytokinin oxidase activity (Taj ALdeen & SAbd El-Aal, 2021).

In recent years, the application of synthetic cytokinins such as 6-Benzylaminopurine (BAP), Kinetin, and Thidiazuron (TDZ) has significantly improved micropropagation techniques for ornamental plants. These growth regulators stimulate bud formation, enhance axillary shoot proliferation, and increase shoot multiplication rates (Ahmad et al., 2020; Rout et al., 2021). TDZ has gained attention due to its vigorous cytokinin-like activity, even at low concentrations, and its

effectiveness in inducing adventitious shoot regeneration, particularly in species considered difficult to propagate (Al-Khayri & Naik, 2021). Recent studies have shown that TDZ performs better than traditional cytokinins in promoting shoot proliferation of *Philodendron*, *Spathiphyllum*, and *Anthurium*, (Nguyen et al., 2020). However, studies on the application of TDZ in *Aglaonema* remain scarce, especially for high-value cultivars such as 'Ruby,' which are increasingly popular in floriculture and landscaping. This current study which assesses the efficacy of TDZ in increasing the microshoot multiplication of *Aglaonema* 'Ruby,' will establish a scientific foundation for large-scale in vitro propagation and microfloriculture development.

This research aimed to examine the influence of the type and concentration of cytokinin on the multiplication of *Aglaonema* 'Ruby' microshoots and to determine what type of cytokinin and at what concentration is most effective in stimulating the multiplication of *Aglaonema* 'Ruby' microshoots. This research is expected to accelerate and increase the production of the *Aglaonema* 'Ruby' shoot to support both mass production of seedlings and microfloriculture products.

MATERIALS AND METHODS

Plant Material

The plant material used was Aglaonema 'Ruby' microshoots, the culture collection of Plant In vitro Culture Laboratory Faculty of Biology, Jenderal Soedirman University. The culture was maintained on Murashige-Skoog medium (PhytoTech M519) supplemented with 20 g.L^{-1} sucrose, 15 µM BAP (Sigma-Aldrich B3408), and solidified with 2.5% phytagel (P8169). The explants were prepared by cutting the apical microshoot at 2 cm long. The apical microshoots were cultured on MS media without a growth regulator and incubated at 24°C under continuous light for 12 days to obtain explants at the same growth phase.

Microshoot Multiplication

The study has been conducted experimentally using a split-plot design with three replications. The main plots were the types of cytokinin consisting of BAP (Sigma-Aldrich B3408), Kinetin (Sigma-Aldrich K0753), and Thidiazuron (Sigma-Aldrich P6186), while the subplots were cytokinin concentrations consisted of 0 µM; 5 µM; 10 µM; 15 µM and 20 µM. The medium used was MS medium (PhytoTech M519) supplemented with 20 g.L^{-1} sucrose and solidified with 2.5 g.L^{-1} phytagel. The microshoots were cultured (1 explant bottle^{-1}) and incubated at 24°C under continuous light for 12 weeks. The variable observed was the growth of *Aglaonema* 'Ruby' microshoots, as measured by number of shoots, shoot length, and number of leaves, which were measured after 16 weeks of culture. Shoot length measurements were carried out by placing the explants on sterile millimeter blocks, and the difference between the initial and the final shoot length was then calculated. The data obtained were subsequently used for Relative Growth Rate (RGR) calculations. RGR was calculated according to Hunt (1990) using the following equation.

$$RGR = \frac{(\ln D_2 - \ln D_1)}{(t_2 - t_1)} \qquad (1)$$

where *D* is parameter data measured at respective time and t is time at two times interval, t_1 and t_2.

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Data Analysis

The data obtained were analyzed with an Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a 95% confidence level using DSAASTAT VER 1.514 software.

RESULTS AND DISCUSSION

The ANOVA results on the effect of cytokinin types and concentrations on *Aglaonema* 'Ruby' microshoots multiplication (Table 1) showed that the type of cytokinin very significantly affected the number of shoots formed and its corresponding RGR, and significantly affected shoot height and its RGR. Furthermore, Table 1 also showed that cytokinin concentrations significantly affected the average number of shoots and RGR of shoot number. Meanwhile, the interaction between type and concentration of cytokinin only significantly affected the RGR number of shoots. In addition, the treatments did not affect the number of leaves and their corresponding RGR. These findings were consistent with Prasetyo et al. (2020), who stated that adding a plant growth regulator would increase plant growth. Smeringai et al. (2023) added that cytokinin application increased the number of lateral shoots. Kieber & Schaller, (2018); Prasad, (2022) mentioned that cytokinin speeds up the cell cycle's mitosis phase, resulting in faster cell differentiation and subsequent shoot formation.

Table 1. Summary of ANOVA results on the effect of types and concentrations of cytokinin on *Aglaonema* 'Ruby' microshoots growth at 12 weeks after planting

Source of Variance	Parameters					
	Number of Shoots	RGR of number of Shoots	Shoot length	RGR of shoot length	Number of leaves	RGR of number of leaves
p-Type of cytokinin	0.0029**	0.0023**	0.0266*	0.0491*	0.23970	0.20670
p-Cytokinin concentration	0.0029**	0.0001**	0.0993	0.2737	0.19160	0.20990
p-Interaction between type and concentration of cytokinin	0.1426	0.0303*	0.3059	0.4938	0.08890	0.09680

Note: ** and * are statistically different at F test 99% and 95%, respectively

The results of Duncan's multiple range test (DMRT) on the effect of cytokinin types on the average number of Aglaonema 'Ruby' microshoots at 12 weeks after planting (Figure 1) showed that TDZ application resulted in the highest shoot number (6.00 shoots. explant-1). This result significantly different from that of BAP and Kinetin applications. The smallest average number of shoots was produced by Kinetin (3.07 shoots.explant-1), although it was not significantly different from the BAP treatment (3.20 shoots. explant-1). Furthermore, RGR calculation also showed consistent results. The DMRT results (Figure 2) showed that TDZ resulted in the highest RGR (0.135 shoots.week-1), significantly different from those produced by BAP and Kinetin. Eventhough the TDZ application resulted in the highest number of shoots and RGR, the size of shoots produced was much smaller (5-9 mm long); in contrast, BAP and Kinetin applications produced shoots > 10 mm long (Figure 3). Schuchovski et al., (2020) reported that TDZ application at the right concentration can increase explant growth by forming shoot apical meristem (SAM) or shoot. Zahara & Win (2020) suggested that combining TDZ with auxin

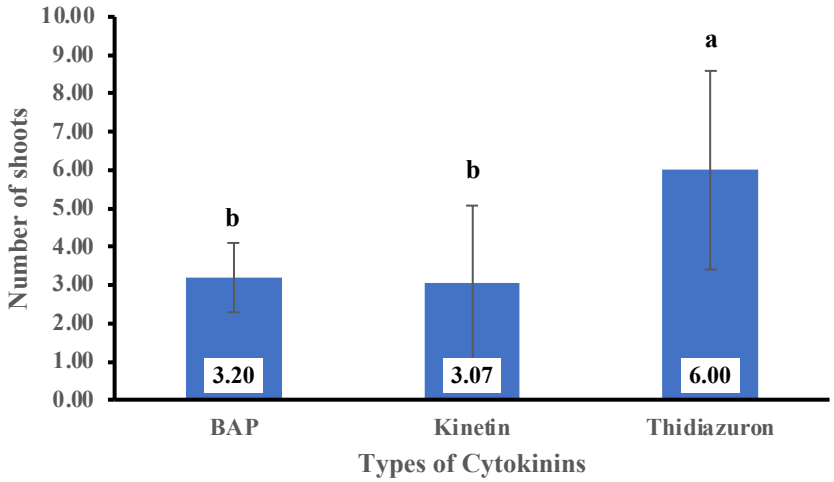

Figure 1. The effect of cytokinin types on the average number of *Aglaonema* 'Ruby' microshoots at 12 weeks after planting (n = 15). Note: a,b—Means marked with different letters in the same line are statistically different at DMRT 95%

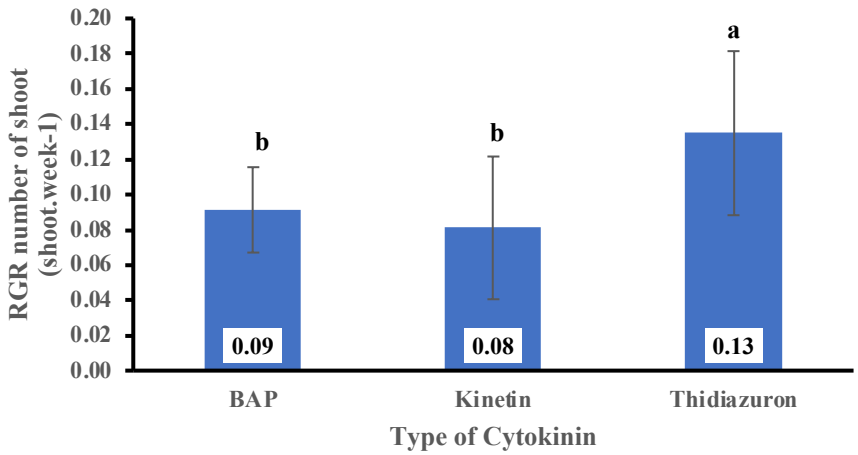

Figure 2. The effect of cytokinin types on the average RGR of *Aglaonema* 'Ruby' microshoots number at 12 weeks after planting (n = 15). Note: a,b—Means marked with different letters in the same line are statistically different at DMRT 95%

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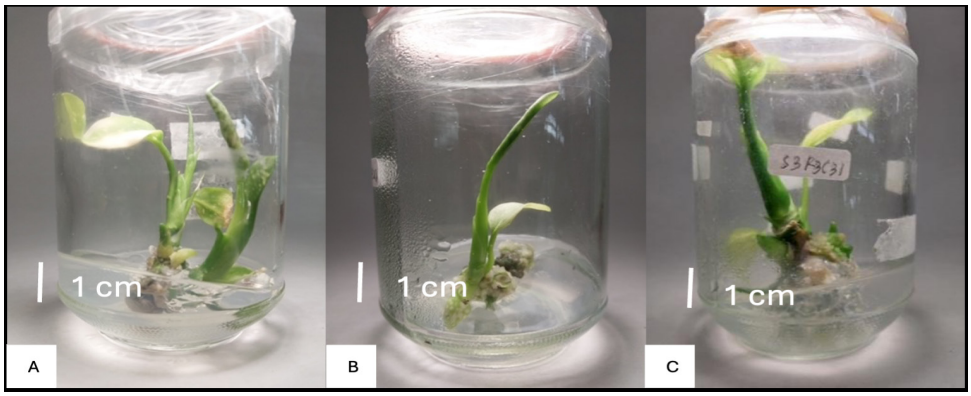

Figure 3. *Aglaonema* 'Ruby' microshoots cultured on MS media supplemented with different types of cytokinin at the same concentration (10 µM) after 12 weeks of culture. Note:(a) BAP, (b) Kinetin, (c) Thidiazuron.

at low concentrations was very effective in inducing shoot formation of *Aglaonema* sp. CV. Lady Valentine, with an average shoot, formed 10.9 shoots.explant^{-1}.

DMRT results on the effect of cytokinin concentrations on the average number of *Aglaonema* 'Ruby' microshoots and its corresponding RGR (Figures 4 & 5) showed that the addition of cytokinin at a concentration of 10 µM resulted in the most number of shoots formed (6.333 shoots. explants^{-1}; Figure 4) and highest RGR of the number shoot (0.142 shoots.week^{-1}, Figure 5), which were significantly different to all cytokinin concentrations tested. Thidiazuron at 10 µM resulted in the highest number of shoots compared to BAP and Kinetin (Figure 3). The shoot's appearance under different concentrations of TDZ is shown in Figure 6. Figure 6 showed that 10 µM produced more shoots than other TDZ concentrations. This result is in accordance with Hai et al. (2020) and Wu et al. (2021), who reported that using cytokinin at the right concentration can increase the number of lateral shoots formed. Furthermore, Klomkamhaeng et al. (2019) showed that a combination of 2 mg.L^{-1} TDZ (equivalent to 9.08 µM) was able to produce 5.40 shoots/explant and 7.30 leaves/explant in *Anubias congensis* multiplication.

Data in Figures 4 & 5 also showed that the smallest average number of shoots and its RGR were demonstrated by the concentration of 0 µM with an average number of shoots formed 2.00 shoots.explant^{-1} and an RGR of 0.057 shoots.week-1. These results indicated that cytokinin is needed to optimally induce the formation of lateral shoots of *Aglaonema* 'Ruby'. These results were in line with Nowakowska et al. (2022), who showed that MS0 media (without cytokinin) produced an average of 0.9 shoots.explants^{-1}, in contrast to MS media supplemented with TDZ at 1 mg.L^{-1} resulted in an average of 2.6 shoots.explants^{-1} in Rhododendron' Kazimierz Odnowiciel' culture. Kaviani et al. (2019) also reported that using MS0 media (control) could only produce 1.00 shoots.explants^{-1} in *Aglaonema* sp. cv. Widuri culture.

The DMRT results of the average RGR of shoot number in response to the interaction between the type and concentration of cytokinin given (Figure 7) showed that the highest number of shoots was obtained from explants grown on MS media supplemented with 10 µM TDZ (RGR of 0.180 shoots. week^{-1}). However, it was not significantly different from those produced by TDZ

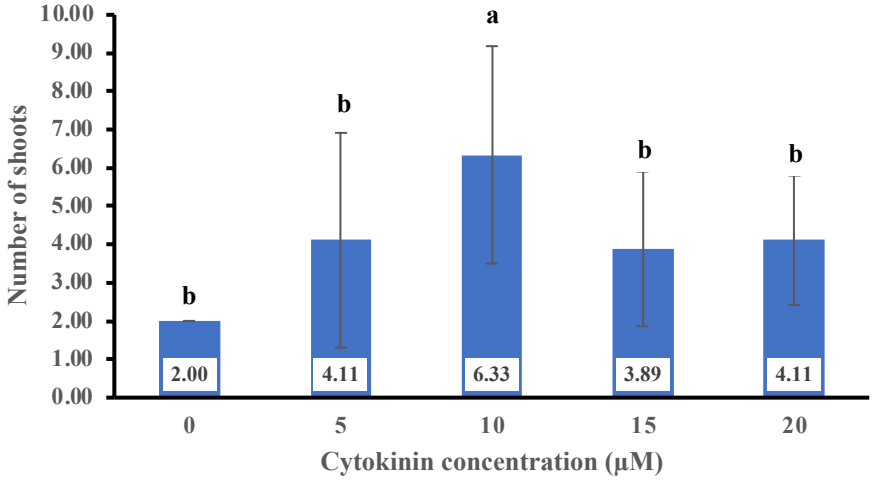

Figure 4. The effect of cytokinin concentrations on the average number of *Aglaonema* 'Ruby' microshoots at 12 weeks after planting (n = 9). Note: a,b Means marked with different letters in the same line are statistically different at DMRT 95%

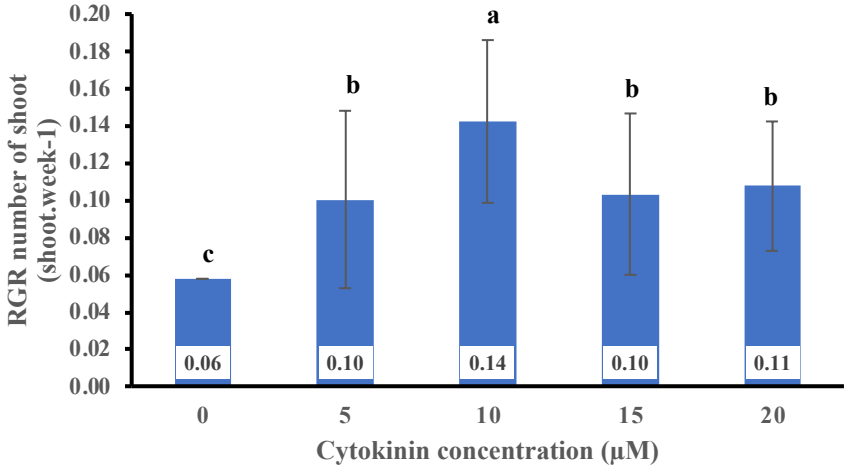

Figure 5. The effect of cytokinin concentrations on the average RGR of *Aglaonema* 'Ruby' microshoot number at 12 weeks after planting (n = 9). Note: a,b—Means marked with different letters in the same line are statistically different at DMRT 95%

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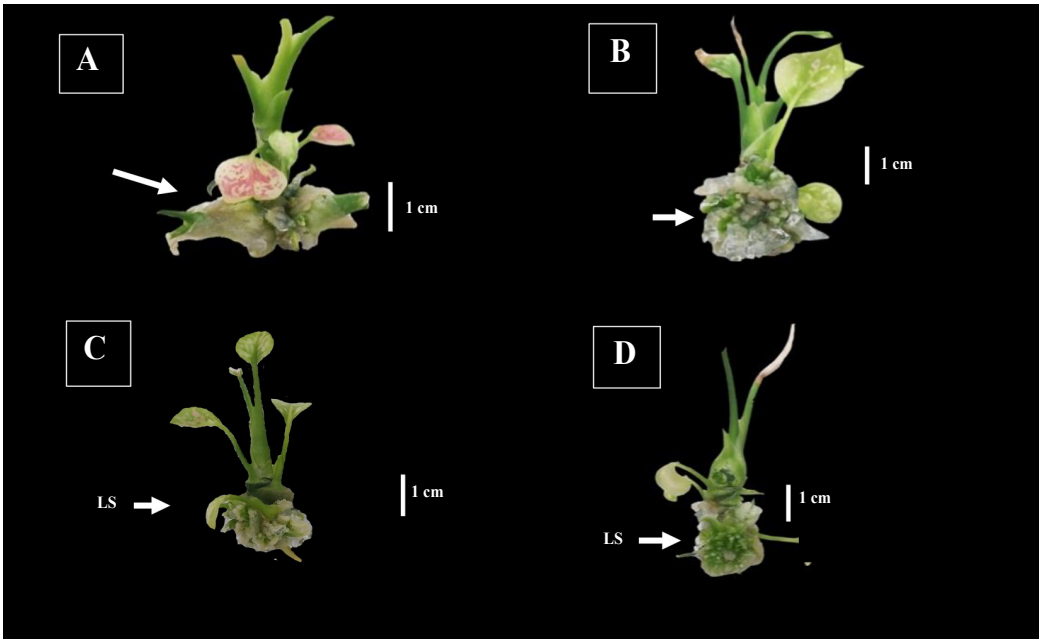

Figure 6. Growth of *Aglaonema* 'Ruby' shoots at different Thidiazuron concentrations. Note: (A) 0 µM, (B) 5 µM, (C) 10 µM, (D) 15 µM; LS (lateral shoot)

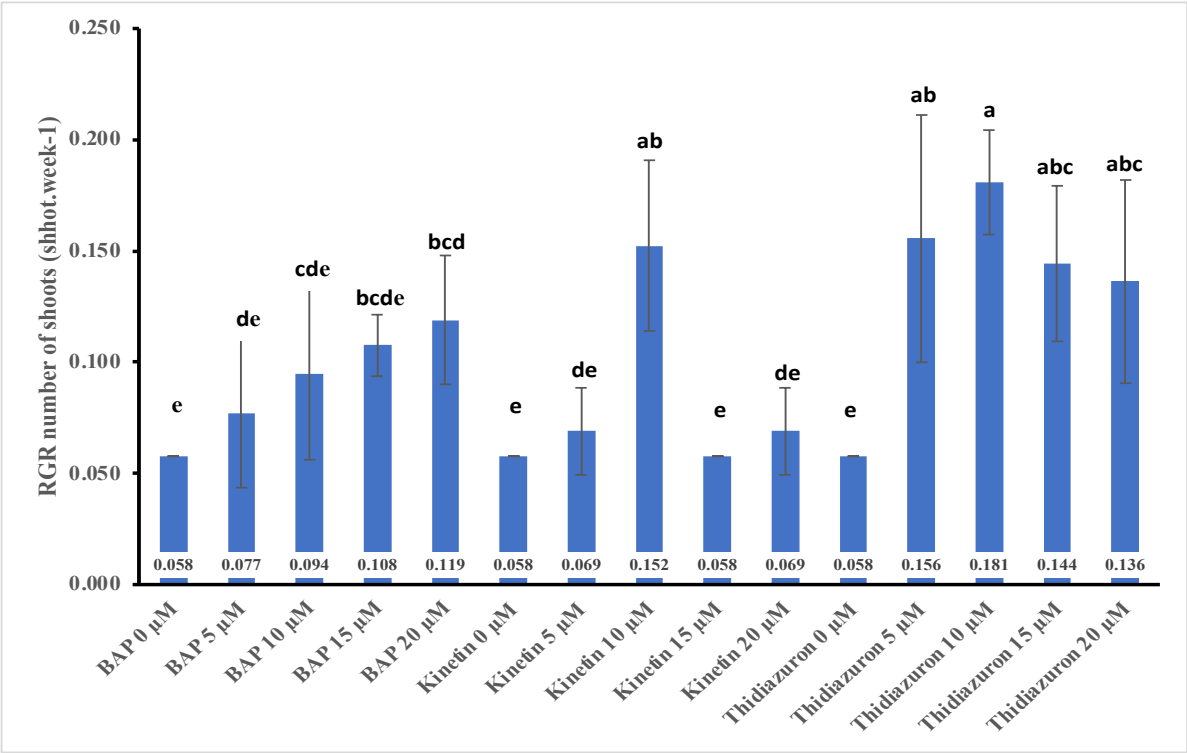

Figure 7. The effect of the interaction between types and concentrations of cytokinin on the average RGR of *Aglaonema* 'Ruby' microshoot number at 12 weeks after planting (n = 3). Note: The numbers on the graph show the relative growth rate time per week. a,b,c,d,e—Means marked with different letters in the same line are statistically different at DMRT 95%

5 µM, TDZ 15 µM, and Kinetin 10 µM. Tuncer (2021) reported that TDZ effectively induced lateral shoot formation on various plants. Dinani et al. (2018) stated that shoot induction stimulated by TDZ begins with the cytokinin ribonucleotides changing into biologically more active ribonucleosides. Ahmad & Faisal, (2018) reported that using TDZ at concentrations of 1 µM to 10 µM was optimum for increasing the number of shoots, shoot regeneration, callus induction, number of nodes, and embryogenic callus induction in vitro on various plants.

Several treatments, including BAP 0 µM, Kinetin 0 µM, Kinetin 15 µM, and TDZ 0 µM, showed the lowest rate of shoot formation with an average RGR of 0.057 shoots.week^{-1}. These results were not significantly different from those produced by BAP 5 µM, BAP 10 µM, BAP 15 µM, Kinetin 5 µM, and Kinetin 20 µM. The RGR of shoots under BAP and Kinetin treatments did not produce optimal results, possibly due to high levels of endogenous cytokinin in the explants and habituation that occurred on the explants used. The explants had previously been cultured under 15 µM BAP for an extended period, suggesting that endogenous cytokinin build up may have occurred, leading to habituation (Tamyiz et al., 2022). BAP is a plant growth regulator that can easily be combined with sugar and stored in the form of 3,β-D-Glucopyranosyl-BAP (3G-BAP) and 9,β-D-Glucopyranosyl-BAP (9G-BAP). The enzyme β-Glucosidase can hydrolyze these conjugates to produce free BAP, which can be transported and used by plants (Mishra et al., 2022; Wang, Gourrierec et al., 2021; Wang, Su et al., 2021). This free BAP is known to promote shoot growth, but excessive concentrations can inhibit explant growth and even have herbicidal effects (Gethami & Sayed, 2020). Cytokinin addition to the media is crucial for shoot multiplication. The balance between cytokinin and auxin control shoot induction, and the variations of auxin and cytokinin concentrations affect shoot initiation (Jing & Strader, 2019).

Recent studies have shown that BA promotes shoot growth in *Aglaonema* species more effectively than TDZ. BA at a concentration of 2.0 mg.L^{-1} considerably increased shoot multiplication in *Aglaonema* Lady Valentine, surpassing TDZ treatments (El-Gedawey & Hussein (2022). Sawardekar et al., (2024) discovered the ideal BA concentration for shoot induction in *Aglaonema* var. Red Valentine was 1.5 mg.L^{-1} higher concentrations resulted in lower-quality shoots and more callus development.

BAP, a synthetic adenine-derived cytokinin, facilitates shoot organogenesis by enhancing cell division and activating cytokinin signaling pathways. It enhances the expression of type-A response regulators and genes associated with the shoot apical meristem, including WUSCHEL and CYCD3, which are essential for meristem preservation and shoot development (Li et al., 2022; Nguyen et al., 2021). Moreover, TDZ, a phenylurea-derived cytokinin-like molecule, has higher cytokinin activity and can drastically affect endogenous hormone balance. TDZ not only inhibits cytokinin oxidase/dehydrogenase (CKX), resulting in the accumulation of endogenous cytokinins but also disrupts auxin metabolism, thereby altering the auxin-to-cytokinin ratio, which is a critical factor in determining organogenic versus embryogenic responses (Nisler et al., 2021; Wang et al., 2025). At low doses, TDZ can promote shoot induction; however, greater concentrations frequently favor callus development or somatic embryogenesis because of its powerful reprogramming effects on cellular differentiation pathways.

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The DMRT results on the effect of cytokinin concentration on the average shoot length (Figure 8) and its corresponding RGR (Figure 9) showed that the longest *Aglaonema* 'Ruby' microshoot was observed on the explant grown in TDZ-containing media (58.20 mm.explant^{-1}). TDZ application also resulted in the fastest shoot elongation with RGR of 0.081 mm.weeks^{-1}. However, these results were not significantly different from those of Kinetin (44.53 mm.explant-1 with an RGR of 0.063 mm.week^{-1}). Taha et al. (2021) also reported that the use of TDZ in culture can stimulate different responses in each culture, depending on the interaction between TDZ and endogenous growth regulators in the explants. Ahmad & Faisal (2018) reported that TDZ application can optimize endogenous auxins/cytokinins ratio during somatic embryogenesis induction by forming auxin-like bioregulators. TDZ can induce better shoot elongation compared to other types of cytokinin. In contrast, Restanto et al. (2024) stated that the media supplemented with 8 mg.L^{-1} BAP showed the best results with the fastest initial explant response at 10.8 day after planting (dap), the highest number of shoots at 2 shoots.explants^{-1}, and the highest shoot at 0.75 cm in the culture of *Aglaonema commutatum* Schott.

The ANOVA results (Table 1) also showed that all the treatments tested did not significantly affect the number of leaves. This condition might be due to the high concentration of cytokinin in the explants, which causes continuous shoot formation, which resulted in the inhibition of axillary shoot elongation and subsequent leaf formation (Dewir et al., 2018). Another factor inhibiting leaf formation is exposure to high cytokinin concentrations for extended periods. The explants used have been incubated on 15 µM BAP from the culture initiation and maintenance period. High cytokinin concentrations can inhibit shoot development (Neil Emery & Kisiala, 2020), elongation, and proliferation (Sakakibara, 2021). Hussain et al. (2021) and Wu et al. (2021) reported that the interaction between cytokinin and auxin strongly influences the initiation of leaf primordia, while the position of leaf primordia is influenced by auxin transport.

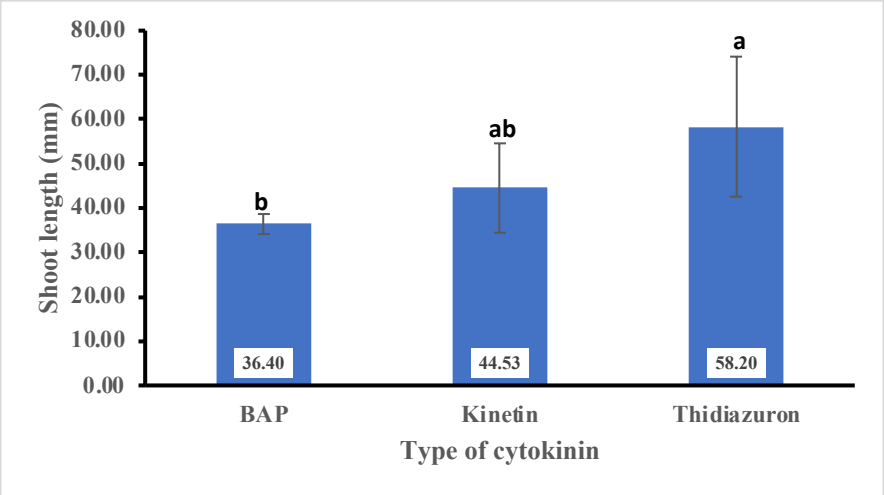

Figure 8. The effect of cytokinin types on the average length of *Aglaonema* 'Ruby' microshoots at 12 weeks after planting (n = 15). Note: a,b—Means marked with different letters in the same line are statistically different at DMRT 95%

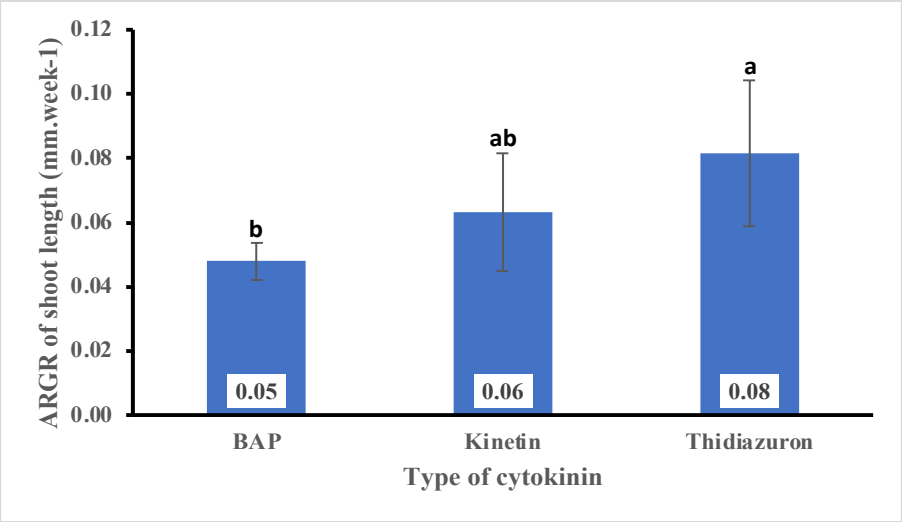

Figure 9. The effect of cytokinin types on the average RGR of Aglaonema 'Ruby' microshoots length at 12 weeks after planting (n = 15). Note: a,b—Means marked with different letters in the same line are statistically different at DMRT 95%

Based on the parameters measured, it was found that TDZ was the most effective cytokinin in inducing microshoot growth, as shown by the number of shoots, shoot length, and their respective RGRs. TDZ is a class of non-adenine cytokinin derived from phenylurea derivatives that are more active than other types of cytokinins (Brunoni et al., 2021; L. Li et al., 2022). TDZ can optimize endogenous growth regulators present in explants (Erland et al., 2020), can promote shoot induction by stimulating cell division and multiplication in the apical meristem and rejuvenating cells to the developmental stage (Nisler et al., 2021; Ricci & Rolli, 2020).

The absence of a significant interaction between BA and TDZ suggests that their effects on shoot induction are independent and additive rather than synergistic or antagonistic. It also indicates that each cytokinin operates through distinct pathways or mechanisms without influencing the efficacy of the other within the tested concentration ranges. Similar observations have been reported in previous studies. Legesse et al., (2022) found that both BA and TDZ individually enhanced shoot proliferation in *Piper nigrum*, with BA alone yielding a higher number of shoots than combined treatments, implying independent action. These findings support the notion that BA and TDZ can function additively, providing flexibility in optimizing cytokinin concentrations for efficient shoot induction.

TDZ has cytokinin-like (CK-like) activity that has been shown to initiate and proliferate shoots (Ahmad & Faisal, 2018; Naaz et al., 2021; Yang et al., 2021). TDZ can modulate the cytokinin biosynthesis pathway by masquerading as an endogenous cytokinin of the adenine class (Ahmad & Faisal, 2018). Furthermore, TDZ is believed to be able to convert cytokinin ribonucleotides (inactive cytokinin) into cytokinin ribonucleosides (active cytokinin) by increasing purine synthesis and inhibiting cytokinin degradation (Bidon et al., 2020; Kaur et al., 2022; Powell & Heyl, 2023). Erland et al. (2020) reported that TDZ could increase the affinity of cytokinin receptors such as cytokinin response 1 (CRE1) and cytokinin response 1/ Arabidopsis histidine kinase 4 (CRE1/AHK4), Arabidopsis histidine kinase 2 (AHK2) and

Arabidopsis histidine kinase 3 (AHK3). In addition, Brunoni et al. (2021) reported that phenylurea-derived cytokinin shows an increased affinity for Cytokinin-specific binding proteins (CSBPs). According to Nisler (2018), TDZ can increase endogenous cytokinin concentrations by reducing its catabolism by inactivating cytokinin oxidase (CKX), increasing synthesis, and converting cytokinin molecules from inactive to active.

Recent studies have also shown that TDZ, significantly enhances the micropropagation efficiency of *Aglaonema* species. In *Aglaonema* Lady Valentine, the Murashige and Skoog (MS) medium supplemented with 2.0 mg.L^{-1} TDZ resulted in the highest shoot proliferation response, with average shoot lengths reaching 4.98 cm (El-Gedawey & Hussein, 2022). Similarly, in *Aglaonema commutatum*, a combination of 2.0 mg.L^{-1} TDZ and 0.5 mg.L^{-1} 1-Naphthaleneacetic acid (NAA) markedly improved both shoot and root development, yielding mean lengths of 8.5 cm for shoots and 7.33 cm for roots (Taj ALdeen & SAbd El-Aal, 2021). In *Aglaonema simplex*, the application of 2.5 mg.L^{-1} TDZ combined with 0.25 mg/L NAA produced the highest number of shoots per culture, averaging 4.49 shoots with a mean shoot length of 1.60 cm after 8 weeks of culture (Soontornyatara & Klammorn, 2020). These findings indicated that TDZ concentrations between 2.0 and 2.5 mg.L^{-1}, particularly when paired with low levels of auxins like NAA, are optimal for in vitro culture of *Aglaonema* species, promoting effective shoot proliferation and overall plantlet development.

The consistent and linear effects of BA and TDZ across the studied concentration ranges and the absence of significant interactions between them indicate that these cytokinins promote shoot growth independently. This finding has dramatically influenced the standardization and scalability of in vitro propagation procedures. The independent action of these cytokinins simplifies medium formulation, as their concentrations can be optimized separately without the need for complex synergistic calibrations. For commercial micropropagation systems, this predictability enhances repeatability, which is essential, especially in the decorative plant sector, where large-scale production demands uniformity, efficiency, and cost-effectiveness. It has also been demonstrated in recent studies on *Aglaonema* spp., a high-value ornamental foliage plant widely propagated through tissue culture. El-Gedawey & Hussein, (2022) reported that BA and TDZ significantly enhanced shoot multiplication in *Aglaonema* Lady Valentine when used individually. Their combined application did not yield superior results, supporting the notion of their independent and non-synergistic effects. Similarly, Sawardekar et al., (2024) emphasized the efficiency and stability of optimized cytokinin concentrations in mass-propagating *Aglaonema* var. Red Valentine highlighting the feasibility of scale-up using simplified media formulations. These findings underscore the utility of the current results for establishing robust, efficient, and industry-relevant micropropagation protocols.

CONCLUSION

It can be concluded that the growth of *Aglaonema* 'Ruby' microshoots was controlled by the type and concentration of cytokinin given. Thidiazuron was better than Kinetin and BAP in stimulating the growth of *Aglaonema* 'Ruby' microshoots. Cytokinin at 10 µM seemed to be effective in improving *Aglaonema* 'Ruby' microshoots multiplication. TDZ at 10 µM can increase

the production of *Aglaonema* 'Ruby' shoot to support both mass production of seedlings and microfloriculture products. Further studies are needed to optimize shoot and root development to produce good plantlets, which will ease the subsequent acclimatization.

AUTHOR CONTRIBUTION

A.A.P. has been responsible for conducting the lab work and preparing the first draft. **R.P.** and **S.S.** responsible for preparing the draft. S has been responsible in managing the whole project, finalising the draft, and doing the submission and correspondence.

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CONFLICT OF INTEREST

No conflict of interest occur regarding the research or the research funding

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