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Effect of meta-topolin and kinetin at various concentrations on shoot multiplication in white turmeric (*Curcuma zedoaria* Rosc.)

Abstract. White turmeric is a medicinal plant widely used to treat various diseases. The limited availability of white turmeric seedlings is influenced by the scarcity of quality seeds due to the long dormancy of the rhizomes and the high incidence of pathogen attacks. Tissue culture techniques are one alternative to address the problem of white turmeric seedling availability. This study aims to determine the best concentrations of meta-topolin and kinetin for in vitro shoot multiplication of white turmeric. The research was conducted from January to June 2022 at the tissue culture laboratory, Faculty of Agriculture, Universitas Padjadjaran. This study used a completely randomized design consisting of 7 treatments: control (0 mg L^{-1}), meta-topolin (1 mg L^{-1}; 2 mg L^{-1}; 3 mg L^{-1}), kinetin (1 mg L^{-1}; 2 mg L^{-1}; 3 mg L^{-1}). Analysis of variance (ANOVA) and Duncan's multiple range test were used to analyze the statistical effects of meta-topolin and kinetin application. Applying 1 mg L^{-1} meta-topolin yielded the best results for shoot number, indicating that 1 mg L^{-1} meta-topolin is a potential cytokinin for the propagation of *Curcuma zedoaria* Rosc.

Keywords: *Curcuma zedoaria* Rosc. · Kinetin · Meta-Topolin

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Introduction

White turmeric (*Curcuma zedoaria* Rosc.) is a rhizomatous medicinal plant belonging to the Zingiberaceae family, widely used to treat various diseases (Hashiguchi et al., 2022). Turmeric holds significant value as a biopharmaceutical commodity in Indonesia and is primarily used in the formulation of herbal medicines. These species have long served as a cornerstone of traditional Indonesian treatments due to their rich therapeutic properties and diverse active compounds (Rahmat et al., 2021). The rhizome of white turmeric plays a crucial role in treating diseases such as diarrhea, gout, hypertension, and also has properties as an anticancer, antibacterial, antidiabetic, and neuroprotective agent (Hashiguchi et al., 2022). The compounds found in the rhizome of white turmeric include flavonoids, flavonols, stigmasterol, chalcones, and β-sitosterol (Hashiguchi et al., 2022).

In Indonesia, turmeric farming is widespread across nearly all regions, covering a total harvested area of 7,481,396 hectares. East Java stands out as the region with the largest turmeric output (117,108,216 tons), followed by Central Java (25,747,866 tons) and West Java (4,183,745 tons) (Central Bureau of Statistics, 2020), indicating fluctuating productivity.

The challenge of reliance on imported medicinal raw materials in Indonesia's pharmaceutical production stems from the limited availability of local simplicia. This issue is particularly evident with white turmeric, where the limited availability of quality seedlings — only accessible once a year during the rainy or dry season — hampers its cultivation. While white turmeric can be propagated vegetatively through rhizomes, Indonesian farmers have not widely adopted this method. As a result, the price remains high, reaching 90,000 IDR/kg in 2021. Additionally, the long dormancy period of 7-8 months for rhizomes, necessary for full maturity (Krishna et al., 2020), further complicates the availability of seedlings and impacts production.

The limited availability of white turmeric seedlings is partly due to pathogen attacks, including yellow mold (*Aspergillus flavus*) (Krishnakumar et al., 2021), rhizome rot (*Pseudomonas fluorescens*) (Prabhukarthikeyan et al., 2017), and bacterial wilt (*Ralstonia solanacearum*) (Seow-neng et al., 2017). One solution to this problem is tissue culture. Tissue culture is a propagation method that isolates plants aseptically to produce fast-growing, disease-free plants (Espinosa-Leal et al., 2018). This technique is based on the theory of cellular totipotency, which holds that each cell has the ability to grow into a complete plant, genetically identical to the parent (Su, et al., 2021).

The success of plant propagation and development is highly dependent on the growing medium and plant growth regulators (PGRs), one of which is cytokinin. Cytokinins are a group of plant hormones that play a role in cell division, such as promoting lateral bud growth, stimulating stomatal formation, inhibiting leaf senescence, encouraging leaf expansion, and promoting chloroplast development (Wybouw & De Rybel, 2019). Cytokinins that can be used to stimulate shoot formation in white turmeric include meta-topolin and kinetin.

According to the study by Waman et al. (2021), applying meta-topolin at a concentration of 1 mg L^{-1} efficiently promotes shoot growth in *Curcuma mangga* plants. The activity of meta-topolin is effective in delaying leaf senescence and significantly reduces the rapid decomposition of chlorophyll in leaf segments, thereby maintaining higher total chlorophyll content during senescence (Işlek, 2021). Additionally, the application of meta-topolin has been associated with increased protein retention, further contributing to delayed senescence. In comparison with benzyladenine (BA), meta-topolin demonstrates several advantages, including a reduced tendency to induce vitrification and better support for overall plant health and rooting efficiency.

The application of kinetin at concentrations of 2 mg L^{-1} and 1 mg L^{-1} NAA is optimal for shoot regeneration in *Kaempferia angustifolia* Roscoe (Haque & Ghosh, 2018). However, the application of cytokinin at very low concentrations is unable to enhance cell division activity for shoot growth. In contrast, excessively high concentrations fail to promote growth as they inhibit the shoot formation process. This study evaluates the efficacy of meta-topolin and kinetin at different concentrations for in vitro shoot induction of white turmeric. The goal is to identify the most effective cytokinin treatment and concentration for shoot regeneration, develop an efficient micropropagation protocol, and increase seedling availability to support sustainable cultivation of this valuable medicinal plant.

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Materials and Methods

Time and Place. The experiment was conducted from January to June 2022. The experiment occurred in the Tissue Culture Laboratory of Seed Technology, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor.

Materials. The planting material used was white turmeric rhizome shoots obtained from the Biofarmaka Cultivation Conservation Unit (UKBB), Tropical Biofarmaka Study Center, Research and Community Service Institute, IPB University.

Medium Composition. The in vitro culture medium consisted of Murashige and Skoog (MS) medium, agar Gelzan, sucrose, aquades, and cytokinin-type PGR (meta-topolin and kinetin). Other materials used outside the medium preparation included 70% alcohol, 1 N NaOH, 1 N HCl, *n*-hexane, ethyl acetate, ethanol, methanol, acetone, quercetin solution, 10% $AlCl_3$, 1 M CH_3COONa, and 1 M CH_3COOK.

Methods

Experimental design. The method used was an experimental method with a Completely Randomized Design (CRD), consisting of 7 treatments and 4 replications. The treatments included a control (0 mg L^{-1}), three concentrations of meta-topolin (1 mg L^{-1}, 2 mg L^{-1}, and 3 mg L^{-1}), and three concentrations of Kinetin (1 mg L^{-1}, 2 mg L^{-1}, and 3 mg L^{-1}). The PGR treatments were added during the preparation of the culture medium, by incorporating the specified concentrations of meta-topolin or kinetin into the Gelzan-based MS medium before pouring the medium into culture vessels.

Data analysis was performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. The experiment was carried out in the following steps: (1) sterilization of equipment, (2) preparation of media, (3) preparation of explants, (4) planting of explants, and (5) observations conducted at 12 weeks after planting (WAP), including measurements of shoot number, root number and length, number of leaves, plant height, and fresh weight.

Analysis of total chlorophyll content. The total chlorophyll content was measured after the explants were destroyed, following the method of Batubara et al. (2016). Five milligrams of white turmeric leaves were ground and mixed with 250 ml of n-hexane, then extracted using ethyl acetate as a solvent. The extract solution (100 mg L^{-1}) was dissolved in methanol:HCl:water (90:1:1) and transferred into a microtube. The supernatant was collected and placed into a cuvette, then measured at wavelengths of 663 nm and 645 nm using a UV-Visible spectrophotometer. Total chlorophyll content was calculated using the following formula (Arshad et al., 2023):

$$\frac{total\ chlorophyll\ (mg)}{leaf\ weight\ (g)} = [20.2\ (A645) + 8.20\ (A663)] \times \frac{\frac{Vv}{1000}}{W}$$

Where:
A : Absorbance
V : Volume of chlorophyll extract
W: Weight of leaf

Analysis of total flavonoid content (TFC). The total flavonoid content was measured after the plantlets were extracted, following the method of Naznin et al. (2019). The leaves were ground and extracted using ethanol as a solvent up to the boundary mark. The extract solution was transferred into seven 10 ml volumetric flasks (0 mg g^{-1}, 1.6 mg g^{-1}, 3.2 mg g^{-1}, 4.8 mg g^{-1}, 6.4 mg g^{-1}, 8 mg g^{-1}, 9.6 mg g^{-1}). To each flask, 3 mL of methanol and 0.2 mL of $AlCl_3$ were added and allowed to react with a minute. The solution was then diluted with distilled water and incubated for 40 minutes, and the absorbance was measured at a wavelength of 415 nm using a UV-Visible spectrophotometer. The total flavonoid content in the sample was expressed in QE mg/100 g using the following formula V T Nguyen et al (2020)::

$$\text{TFC (mg QE/100 g)} = \frac{C.V}{M}$$

Where:
C : Concentration of quercetin (mL^{-1})
V : Volume of extract (ml)
M: Mass of extract (g)

Results and Discussion

Number of Shoots. The success of shoot multiplication is characterized by the number of shoots that develop. An increase in the number of shoots correlates with a higher multiplication rate of the plant. A high shoot multiplication rate positively influences the production of seedlings. According to the analysis of variance, the application of 1 mg L^{-1} meta-topolin yielded the highest average number of shoots compared to the other treatments (Table 1). This finding is

consistent with the research by Waman et al. (2021), which demonstrated that applying single meta-topolin (without auxin combination) at a concentration of 1 mg L^{-1} effectively promotes shoot growth in *Curcuma mangga* plants.

According to Gantait, S., & Mitra, M. (2021), meta-topolin (mT) has been shown to not only support the efficient induction of multiple shoots, similar to or better than BA, but also to minimize physiological disorders commonly associated with BA and TDZ, such as hyperhydricity and inhibited rooting. This contributes to improved shoot quality and overall success in the micropropagation of horticultural species. Meta-topolin is also considered more active in shoot multiplication than zeatin, Kinetin, and BA, making it a viable alternative cytokinin to replace BA in inducing shoot formation (Gantait, S., & Mitra, M., 2021).

Meta-topolin is a potent cytokinin effective in stimulating shoot organogenesis across various plant species. It activates cell division receptors (AHK3) with higher affinity than other cytokinins, promoting enhanced shoot formation (Gantait & Mitra, 2021). In cassava, a two-stage protocol with meta-topolin improved shoot regeneration by over 35% in cultivar TME 7 (Chauhan & Taylor, 2018). However, shoot induction by meta-topolin is concentration-dependent, with higher concentrations potentially reducing shoot numbers, such as at 2 mg L^{-1}. These results underscore meta-topolin's potential in promoting shoot organogenesis, though its effectiveness varies with concentration.

Table 1. Differences in shoots number in response to meta-topolin and kinetin application

Treatments	Number of Shoots
A (Control)	0.86 a
B (1 mg L^{-1} meta-topolin)	3.75 b
C (2 mg L^{-1} meta-topolin)	1.19 a
D (3 mg L^{-1} meta-topolin)	1.63 a
E (1 mg L^{-1} kinetin)	1.19 a
F (2 mg L^{-1} kinetin)	1.00 a
G (3 mg L^{-1} kinetin)	1.50 a

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

According to Waman et al. (2021), applying excessively high concentrations of meta-topolin in explants can result in a loss of shoot quality and abnormal shoot development. Increasing cytokinin concentrations may reduce shoot elongation, thus hindering shoot multiplication. Table 1 shows that explants cultured in the control medium, which did not receive any cytokinin application, produced the lowest number of shoots. This is likely due to the insufficient endogenous cytokinin alone to stimulate shoot formation. When exogenous cytokinin was added, it induced the formation of shoots at twice the number compared to the control. The addition of exogenous plant growth regulators, such as cytokinins, can regulate apical dominance by stimulating the formation of axillary shoots. Therefore, when applied at high concentrations, cytokinins can induce shoot formation (Wybouw & De Rybel, 2019).

The number of roots. Roots are essential for the absorption of water and nutrients, and an increased root number enhances the plant's ability to efficiently fulfill its nutritional demands. In environments with abundant nutrients, root systems exhibit morphological adaptations, such as the growth of additional root hairs, which facilitate more effective nutrient uptake (Farhangi-Abriz, S., et al., 2023). These structural modifications in the root system are critical for optimizing nutrient absorption, enabling plants to thrive in diverse conditions. Table 2 shows that applying 3 mg L^{-1} Kinetin resulted in a higher average number of roots, although the difference was not statistically significant compared to the 2 mg L^{-1} Kinetin treatment.

The observed Root growth in explants is primarily driven by high endogenous auxin levels, which are essential for root development, including organogenesis and response to environmental stimuli (Cavallari et al., 2021). Auxin regulates the architecture of the root system, influencing both primary and lateral root formation. Additionally, the presence of cytokinins in explants boosts ethylene production, further promoting adventitious root formation. Wounding during explant preparation stimulates the synthesis of endogenous auxins and other growth regulators, which are critical for root development (Pasternak & Steinmacher, 2024). Together, endogenous auxins and external cytokinins play a vital role in root formation and successful in vitro plant regeneration.

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Table 2. Differences in roots number and length in response to meta-topolin and kinetin application

	Treatments	Number of Roots	Length Roots (cm)
A	(Control)	3.65 a	5.38 a
B	(1 mg L^{-1} meta-topolin)	4.69 a	4.82 a
C	(2 mg L^{-1} meta-topolin)	4.94 a	4.91 a
D	(3 mg L^{-1} meta-topolin)	4.46 a	5.38 a
E	(1 mg L^{-1} kinetin)	4.92 a	6.81 b
F	(2 mg L^{-1} kinetin)	8.25 b	8.59 c
G	(3 mg L^{-1} kinetin)	9.44 b	7.71 bc

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

The analysis of variance results show that the longest root length was observed in the 2 mg L^{-1} kinetin treatment. According to Yasin et al. (2018), kinetin is a type of cytokinin that is crucial in stimulating the apical meristem in cell division and elongation, thereby enhancing root elongation. Kinetin also increases the accumulation of photosynthates in the roots, leading to greater root elongation due to the balance of nutrients across the plant's tissues (Al-Zubaidi et al., 2020). However, in this study, increasing the kinetin concentration did not correspond to an increase in root length. This is likely because the endogenous auxin in the explants was already sufficient to induce root formation and elongation. Thus, exogenous cytokinin application was not required in high concentrations.

The control and meta-topolin treatments did not result in a statistically significant difference in root number or length, indicating that endogenous auxin levels were adequate to support root development. As highlighted by Alaguero-Cordovilla et al. (2021), wounding stimulates localized auxin biosynthesis, which plays a critical role in the initiation of adventitious roots. Root formation is largely determined by the internal concentration of auxin and its synergistic interaction with cytokinins.

The application of meta-topolin at all concentrations resulted in short roots, as observed in the explant appearance at 12 weeks after planting (Figure 1). This is consistent with the findings of Shekhawat et al. (2021) who reported that applying meta-topolin alone is less effective for root induction; however, its combination with auxin significantly improves root characteristics. Combining meta-topolin with auxin is suggested to be more effective for achieving optimal root induction and elongation (Gantait, S., & Mitra, M., 2021).

Number of Leaves. The leaves are an important plant organ as they are directly involved in light-capture processes such as photosynthesis. The number of leaves is a component that indicates plant growth; the more leaves present, the greater the light absorption by the leaves for photosynthesis. Based on the analysis of variance, all treatments did not show a significant difference in the number of leaves (Table 3).

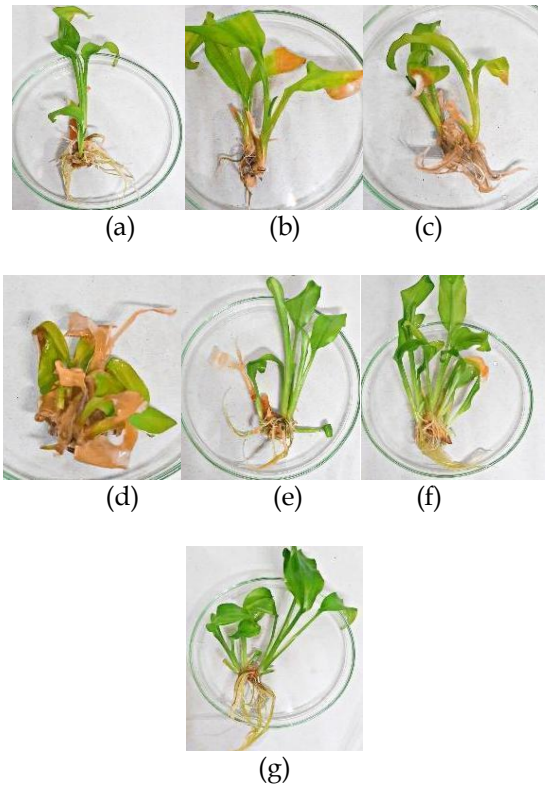

Figure 1. Differences in plantlet morphology at12 weeks after culture: (a) control; (b) 1 mg L^{-1} meta-topolin; (c) 2 mg L^{-1} meta-topolin; (d) 3 mg L^{-1} meta-topolin; (e) 1 mg L^{-1} kinetin; (f) 2 mg L^{-1} kinetin; and (g) 3 mg L^{-1} kinetin

Table 3. Differences in leaves number in response to meta-topolin and kinetin application

	Treatments	Number of Leaves
A	Control)	2.65 a
B	1 mg L^{-1} meta-topolin)	4.94 a
C	2 mg L^{-1} meta-topolin)	3.88 a
D	3 mg L^{-1} meta-topolin)	4.82 a
E	1 mg L^{-1} kinetin)	4.21 a
F	2 mg L^{-1} kinetin)	4.25 a
G	3 mg L^{-1} kinetin)	5.19 a

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Table 3 shows that the number of leaves tended to increase with the application of kinetin, although the increase was not significant. This indicates that although an increase in shoots generally corresponds with an increase in the number of leaves, this is not universally applicable. Leaf development is governed by a combination of hormonal balance and genetic factors, which regulate the function of the shoot apical meristem and leaf initiation. The interaction of these factors can result in variations in leaf production that are independent of the number of shoots (Peng, et al., 2023). Consequently, the application of kinetin to explants did not yield a significant difference in the number of leaves, as there was no substantial variation in the number of shoots produced.

In the meta-Topolin treatment, vitrification symptoms were observed in the leaves of white turmeric with the application of meta-topolin (Figure 1). These symptoms increased with higher concentrations of meta-topolin applied. The application of cytokinins such as BA (benzyladenine) at excessively high concentrations can increase the number of leaves exhibiting vitrification (Gantait, S., & Mitra, M., 2021). Vitrification in plants, particularly in micropropagated species, is a physiological disorder marked by a decrease in stomatal density and an increase in stomatal size, which results in abnormal leaf development. This condition is primarily triggered by high humidity and nutrient-rich culture media that interfere with normal leaf morphology and physiological processes. One of the key features of vitrified leaves is the malfunctioning of stomata, which tend to remain open and unresponsive to environmental cues, thereby causing excessive water loss. This stomatal dysfunction is further exacerbated by structural abnormalities in the cell wall, such as hypolignification and reduced cellulose biosynthesis, which impair stomatal regulation and overall leaf function (Marques et al., 2021).

Plant Height. Plant height is an important component for assessing the response of explants to the treatments applied, as it reflects the growth rate after cultivation. Plant height affects the plant's ability to capture light and the leaf area index. Table 4 shows that the application of 3 mg L^{-1} kinetin resulted in a higher average plant height compared to the meta-topolin treatment. However, this difference was not statistically significant when compared to the control, 1 mg L^{-1} kinetin, and 2 mg L^{-1} kinetin treatments. This is consistent with the study by Gawande et al. (2020), which found that the application of kinetin in *Curcuma longa* significantly resulted in the highest plant height compared to other cytokinin treatments. Kinetin can enhance the activity of both apical and lateral meristems, thereby promoting vegetative growth such as plant height (Khandaker et al., 2018).

Table 4. Differences in plantlet height in response to meta-topolin and kinetin application

	Treatments	Plant Height (cm)
A	Control)	7.12 ab
B	1 mg L^{-1} meta-topolin)	6.11 a
C	2 mg L^{-1} meta-topolin)	6.06 a
D	3 mg L^{-1} meta-topolin)	6.13 a
E	1 mg L^{-1} kinetin)	7.10 ab
F	2 mg L^{-1} kinetin)	7.69 ab
G	3 mg L^{-1} kinetin)	8.15 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

The shortest plant height in Table 4 was observed in explants treated with meta-topolin. The height of the explants is influenced by the number of shoots formed. Based on the number of shoots produced, the explants treated with meta-topolin had a large number of shoots, but the plants were short. As the number of shoots increases, energy and resources are diverted from stem elongation to shoot formation, resulting in a reduction in overall plant height. Wang et al. (2018) suggest that this process is governed by the plant's hormonal balance, which determines whether growth is prioritized for shoot

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development or elongation, thereby influencing the overall growth dynamics.

Fresh weight. Fresh weight refers to the weight obtained from all parts of the plant, including the roots, stems, and leaves. Fresh weight is closely related to vegetative growth; the better the growth, the greater the fresh weight produced. Based on the analysis of variance, the treatment with 3 mg L^{-1} kinetin resulted in the highest fresh weight compared to the other treatments (Table 5).

This is consistent with the study by Chuengpanya et al. (2020), which found that the application of kinetin in *Hedychium longicornutum* plants resulted in the highest fresh weight compared to other cytokinins. Kinetin plays a role in enhancing the efficiency of photosynthesis, which aids in the accumulation of nutrients throughout the plant. As a result, fresh weight increases due to the balanced distribution of nutrients within the plant tissues (Al-Zubaidi et al., 2020).

Table 5. Differences in plantlet fresh weight in response to meta-topolin and kinetin application

	Treatments	Fresh Weight (g)
A	Control)	1.83 a
B	1 mg L^{-1} meta-topolin)	2.68 a
C	2 mg L^{-1} meta-topolin)	1.98 a
D	3 mg L^{-1} meta-topolin)	1.89 a
E	1 mg L^{-1} kinetin)	1.81 a
F	2 mg L^{-1} kinetin)	2.59 a
G	3 mg L^{-1} kinetin)	3.85 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Table 5 shows that the lowest fresh weight of explants was observed in treatment E, where the explants were applied with 1 mg L^{-1} kinetin, resulting in an average fresh weight of 1.81 g. This may be because the exogenous cytokinin application at this concentration is insufficient to achieve maximal fresh weight in the explants. According to Li et al. (2018), an increase in fresh weight in plants is in line with the concentration of kinetin applied. The higher the kinetin concentration applied to the plant, the greater the fresh weight. Therefore, explants treated with 3 mg L^{-1} kinetin were the most effective in producing the highest fresh weight in white turmeric plants.

Total chlorophyll content. Chlorophyll is the green pigment found in chloroplasts, playing a crucial role in photosynthesis. The chlorophyll content in the leaves serves as an indicator of the plant's metabolic balance. According to the analysis of variance, the treatment with 2 mg L^{-1} kinetin resulted in the highest average total chlorophyll content, measuring 0.94 mg g^{-1} (Table 6). This is in line with the findings of Yaowachai et al. (2020), who reported that kinetin helps maintain chlorophyll stability in *Globba globulifera* plants. Li et al. (2018) state that kinetin can preserve chlorophyll stability and enhance antioxidant enzyme activity. The application of kinetin has been shown to improve the formation of photosynthates and increase chlorophyll content in plants (Gurnami et al., 2018).

Table 6. Differences in plantlet chlorophyll content in response to meta-topolin and kinetin application

	Treatments	Total Chlorophyll Content (mg/g)
A	Control)	0.70 b
B	1 mg L^{-1} meta-topolin)	0.58 a
C	2 mg L^{-1} meta-topolin)	0.53 a
D	3 mg L^{-1} meta-topolin)	0.49 a
E	1 mg L^{-1} kinetin)	0.54 a
F	2 mg L^{-1} kinetin)	0.94 c
G	3 mg L^{-1} kinetin)	0.71 b

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Kinetin enhances photosynthetic pigments and chlorophyll content in leaves, increases CO_2 assimilation, boosts photosynthesis rates, and extends the period of active photosynthesis. This occurs because kinetin can promote stomatal conductance and increase the number of chloroplasts in leaves by stimulating cell growth and cytoplasmic activity, thereby increasing chlorophyll synthesis (Khandaker et al., 2018). An increase in kinetin concentration correlates with an increase in the length and width of chloroplasts in plants, meaning that higher kinetin concentrations result in higher chlorophyll content (Li et al., 2018).

Total flavonoid content (TFC). Flavonoids do not directly participate in the normal growth of plants; however, they play an active role in the survival of plants. Flavonoids are a group of polyphenolic compounds with biological effects

such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, and anti-viral properties, and the ability to inhibit cancer development. Based on the variance analysis, the application of 2 mg L^{-1} kinetin, showed the highest average total flavonoid content of 1.21 mg g^{-1} (Table 7). This is in line with the findings of Gantait, S., & Mitra, M. (2021), who reported that kinetin can enhance secondary metabolite content, specifically promoting flavonoids (anthocyanins) in *Haplopappus gracilis* plants. According to Gurav et al. (2020), secondary metabolites are closely related to cell differentiation, meaning that the increase in total flavonoid content highly depends on the plant growth regulators applied.

Table 7. Differences in total flavonoid content in response to meta-topolin and kinetin application

	Treatments	Total Flavonoids Content (mg g^{-1})
A	Control)	1.17 f
B	1 mg L^{-1} meta-topolin)	0.77 e
C	2 mg L^{-1} meta-topolin)	0.46 a
D	3 mg L^{-1} meta-topolin)	0.58 c
E	1 mg L^{-1} kinetin)	0.52 b
F	2 mg L^{-1} kinetin)	1.21 g
G	3 mg L^{-1} kinetin)	1.72 d

Note: Means followed by the same letter and in the same column show no significant difference based on Duncan's test at the 5% level.

Flavonoids are generally plentiful in older leaves and are a main defense mechanism for plants. Flavonoids absorb UV radiation, primarily accumulating in the epidermis of leaves, thus shielding underlying tissues from damage (Ferreyra et al., 2021). This is one of the reasons why the total flavonoid content was assessed in 12-week-old leaves. The high total flavonoid content is also influenced by the conditions in which the plant is grown. According to Yaowachai et al. (2020), in vitro conditions can enhance flavonoid and antioxidant activity in *Globba globulifera*. This occurs because the nutrient and hormone levels remain stable in in vitro conditions, which in turn increases the synthesis and gene expression of secondary metabolites. Table 7 shows that the application of kinetin at the highest concentration (3 mg L^{-1}) did not correlate with an increase in total flavonoid content. This may be due to the fact that the endogenous cytokinin content in the explants was already sufficient to enhance the total flavonoid content in the leaves, meaning that a higher concentration of cytokinin was not necessary.

Conclusion

This study demonstrates that the application of meta-topolin and kinetin at varying concentrations significantly affects the growth of white turmeric explants, influencing key parameters such as shoot and root development, plant height, root length, fresh weight, and the accumulation of total chlorophyll and flavonoids. Among the treatments, 1 mg L^{-1} meta-topolin was the most effective in promoting shoot proliferation, suggesting its potential as an optimal regulator for in vitro propagation. These findings provide valuable insights into the hormonal control of white turmeric explant growth and offer a foundation for enhancing propagation protocols, with important implications for both the sustainable cultivation and commercial production of this medicinal species.

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