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Cytokinin and Auxin Enhance Early Rhizome Development and Essential Oil Production in *Curcuma aromatica* Salisb.

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Abstract

Curcuma aromatica Salisb. is a monocotyledonous plant whose rhizomes contain essential oils with documented medicinal properties. This study evaluated the effects of plant growth regulators (PGRs) at different concentrations on *in vitro* shoot development and essential oil accumulation. A completely randomized design with 17 treatments, including naphthalene acetic acid (NAA, 1.0–2.5 mg·L^{-1}), benzyladenine (BA, 2.0–15.0 mg·L^{-1}), gibberellic acid (GA_3, 5.0–20.0 mg·L^{-1}), Ethephon (5.0–20.0 mg·L^{-1}), and a control, with 10 replicates per treatment, was used. After two weeks, morphological, physiological, and biochemical parameters were measured. Significant increases in bud fresh weight, dry weight, and diameter were observed at NAA (2 mg·L^{-1}), BA (10 mg·L^{-1}), GA_3 (20 mg·L^{-1}), and Ethephon (10 mg·L^{-1}). Among these, NAA (2.0 mg·L^{-1}) and BA (10.0 mg·L^{-1}) were most effective. Compared with the control, NAA (2.0 mg·L^{-1}) increased rhizome diameter (103%), fresh weight (61%), dry weight (327%), sugar content (56%), and essential oil content (143%). BA (10.0 mg·L^{-1}) showed greater effects, increasing rhizome diameter (114%), fresh weight (90%), dry weight (686%), sugar content (80%), and essential oil content (203%) ($p < 0.05$). The number of primary thickening meristem (PTM) cells was significantly higher in NAA (2 mg·L^{-1}) than in BA (10 mg·L^{-1}) or other treatments. Both NAA and BA increased auxin, zeatin, and gibberellin activities, while reducing abscisic acid (ABA) activity compared with the control. These results indicate that NAA and BA enhance rhizome development and essential oil biosynthesis in *C. aromatica* through modulation of endogenous hormones.

Keywords: *Curcuma aromatica* Salisb., cytokinin, auxin, vascular bundles, essential oil yield, growth

1. Introduction

White turmeric *Curcuma aromatica* Salisb., is considered a valuable medicinal plant in traditional Asian medicine, thanks to its antibacterial, antioxidant, anti-inflammatory, and anti-cancer activities [1,2,3]. These biological effects are primarily due to the essential oil and curcuminoid contents in the rhizomes [4]. However, optimizing rhizome yield and enhancing essential oil accumulation under cultivation conditions remain challenging, and therefore it is essential to clarify the anatomical structure and endogenous signals associated with the early development of rhizomes.

In dicotyledonous plants, stem diameter expansion is determined by the vascular cambium – a secondary meristem that arises between the primary xylem and phloem and forms secondary phloem and secondary xylem [5]. Conversely, in monocotyledonous species such as *Curcuma*, radial enlargement of the rhizome is primarily controlled by the primary thickening meristem (PTM)—a specialized meristematic region located between the pith and cortex [6,7,8]. The PTM produces layers of cortical parenchyma, endodermis, dermal tissues, and vascular bundles, thereby contributing directly to radial expansion through coordinated cell division and enlargement of storage parenchyma [9]. Moreover, essential oils accumulate mainly in parenchymatous tissues in white turmeric [10], and PTM activity plays a determining role in influencing rhizome diameter, biomass accumulation, and essential oil storage capacity. Plant growth regulators such as auxin, cytokinin, gibberellin, and ethylene are involved in regulating meristem activity and tissue differentiation, which are especially important in coordinating the early development of organs [11], and may in turn also impact the division of the primary

thickening meristem. In cambial development, auxin plays an important role in cell growth, apical procambium formation, and vascular differentiation [11]. Gibberellin plays a role in cell elongation, leaf development, stimulates cell division in the cambial region, and induces the formation of wood fibers [12]. Cytokinin regulates cambium activity, promotes wood production and secondary growth, and affects cambial division. In transgenic plants, cytokinin signaling decreases simultaneously with a decrease in cell division in the vascular layers and vice versa [11]. Ethylene has the ability to inhibit or stimulate growth and regulate vascular growth, as it can stimulate cambial development in herbaceous plants, and when ethylene biosynthesis inhibitors are used, they also block the differentiation of vascular factors [11]. Although these hormones have been fairly well described in secondary meristems, their specific regulatory role in the activity of the primary thickening meristem (PTM) during the early development phase of the rhizome in *Curcuma* has not been fully explored.

Therefore, this study investigates the effects of four major plant hormones—auxin, cytokinin, gibberellin, and ethylene—on PTM activity, early rhizome enlargement, and essential oil accumulation in *Curcuma aromatica* Salisb., and aims to clarify the mechanisms that underlie early rhizome growth and identify hormonal regulators that could enhance essential oil productivity.

2. Materials and methods

2.1Materials

C. aromatica rhizomes at plant ages of 10–12 months bearing 5–6 resting buds were collected from the Dak Lak Center for Research on Plant and Animal Breeding. The Murashige and Skoog Basal Salt Mixture (MS) was obtained from Sigma-Aldrich (UNSPSC Code: 12352207). Naphthalene acetic acid (NAA) was obtained from Sigma-Aldrich (CAS Number: 86-87-3). Benzyladenine (BA) was obtained from Sigma-Aldrich (CAS Number: 5426-62-0). Gibberellic acid (GA_3) was obtained from Sigma-Aldrich (CAS Number: 77-06-5). Ethephon was obtained from Sigma-Aldrich (CAS Number: 16672-87-0). Distilled water was used for media preparation and chemical dilution.

2.2Methods

2.2.1. Effect of PGRs on the in vitro response of isolated buds of C. aromatica

The resting *C. aromatica* buds were separated from the mother rhizomes, after being sterilized with sodium hypochlorite (NaOCl) 3.5% for 10 minutes, then each bud (10 mm in length) was placed on a cotton MS culture medium supplemented with PGRs at different concentrations. MS cotton culture medium is a liquid Murashige and Skoog medium (10 mL) soaked in a layer of cotton (2 cm thick) placed inside an Erlenmeyer flask. The experiment consisted of 17 treatments: Control (MS without PGR); naphthalene acetic acid (NAA) treatment at concentrations: 1; 1.5; 2 and 2.5 $mg \cdot L^{-1}$; benzyladenine (BA) treatment at concentrations: 2; 5; 10 and 15 $mg \cdot L^{-1}$; gibberellin (GA_3) treatment at concentrations: 5, 10, 15 and 20 $mg \cdot L^{-1}$; Ethephon treatment at concentrations: 5, 10, 15 and 20 $mg \cdot L^{-1}$. Each treatment was repeated 10 times, with three Erlenmeyer flasks per replicate, each flask containing three buds. *In vitro* bud growth after two weeks was determined by fresh weight, dry weight, and rhizome diameter.

2.2.2. Effect of PGRs on physiology and biochemistry changes of C. aromatica buds in vitro

The resting buds of *C. aromatica* were sterilized using the above method and then cultured either on cotton MS medium without PGRs (control) or on MS medium supplemented with 2 $mg \cdot L^{-1}$ NAA, 10 $mg \cdot L^{-1}$ BA, 20 $mg \cdot L^{-1}$ GA_3, or 10 $mg \cdot L^{-1}$ Ethephon. After two weeks, parameters including anatomical structure, PTM layers, essential oil content, number of vascular bundles, sugar and starch contents, and endogenous phytohormone activities were analyzed. The experiment consisted of five treatments, each replicated 10 times, with 10 Erlenmeyer flasks per replicate, and each flask containing three buds. To determine essential oil content from small sample volumes, three series of experiments identical to those described above were conducted.

2.2.3. Analysis of some physiological and chemical changes of C. aromatica rhizomes

2.2.3.1. Determination of anatomy

Rhizome anatomy was observed directly under an optical microscope. Anatomical sections were cut transversely and stained with Lugol's iodine and indigo carmine solutions, and then observed under an optical microscope.

2.2.3.2. Measurement of respiration intensity

Respiration intensity (μmol O_2 g^{-1} FW h^{-1}) of the rhizomes was measured with LeafLab 2 (Hansatech) in the dark.

2.2.3.3. Measurement of tuber diameter, fresh weight and dry weight:

The rhizome diameter was measured with a caliper, at the largest edematous area of the rhizomes, then weighed immediately to obtain fresh weight and dried at 120 °C for 1 hour, then continued drying at 80 °C until the weight remained unchanged (approximately 72 hours) to determine the dry weight.

2.2.3.4. Measurement of total sugar and starch content in rhizomes

One gram of fresh rhizomes with different treatments was ground to extract the total sugar content in ethanol. (1) Place the sample into a 50 mL beaker and add 10 mL of 70% ethanol. Heat the beaker in a boiling water bath for 5 minutes, then filter the extract through filter paper. Repeat this extraction step three times. (2) Afterward, add 10 mL of 90% ethanol to the beaker containing the residue, mix thoroughly, heat in a boiling water bath, and filter again. Perform this step once. (3) Combine all four filtrates and evaporate the solvent to dryness. (4) Reconstitute the dry residue with distilled water to a final volume of 50 mL. The extract was color-reacted with 5% phenol and concentrated sulfuric acid, and the optical density (OD) was measured using a spectrophotometer (Model 1.JPG) at 490 nm. The remaining starch residue was dried and hydrolyzed with perchloric acid. Starch content was determined using a standard curve prepared with glucose solution [13].

2.2.3.5. Analysis of endogenous phytohormones activity

Phytohormones (auxin, cytokinin, gibberellin, and abscisic acid) in rhizomes after two weeks of treatment were extracted with methanol and diethyl ether, then isolated following the procedures described by Hong et al. (2021) [14] and Yokota et al. (1980) [15]. The extracted phytohormones were separated using silica gel thin-layer chromatography (60 F_{254}, 105554, Merck) at 29 °C with a mobile phase consisting of chloroform:methanol:acetic acid (80:15:5, v/v). Spots were visualized under ultraviolet light. Phytohormone activity was assessed using semi-quantitative bioassays, in which auxin activity was measured using lettuce hypocotyls (*Lactuca sativa* L.), cytokinin (zeatin) activity using cucumber cotyledons (*Cucumis sativus* L.), gibberellin activity using rice coleoptile sections (*Oryza sativa* L.), and abscisic acid activity using standard ABA bioassays. All measurements were performed in triplicate.

2.2.3.6. Measurement of essential oil content in rhizomes

The essential oil content in 100 g of fresh rhizomes was determined by steam distillation using a Clevenger apparatus for 4 hours [16].

2.2.4. Statistical analysis

All experimental data were analyzed using SPSS 20.0 for Windows. Differences among treatments were evaluated using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test to determine pairwise differences. Statistical significance was accepted at $p < 0.05$.
For anatomical observations, including changes in the number of vascular bundles and the number of primary thickening meristem (PTM) layers in *Curcuma aromatica* rhizomes after two weeks of plant growth regulator (PGR) treatments, three biological replicates were used, and for each replicate, three technical measurements were performed.

3. Results

3.1 Effect of PGRs on the response of white turmeric buds in vitro

In BA treatment, buds were observed as thick, globular shapes. In the untreated control, NAA, Ethephon, and GA_3, buds had a pyramid shape (Figure 1). The diameter, fresh weight, and dry weight of *C. aromatica* rhizomes after two weeks of treatment increased in all treatments compared to the control. These growth parameters treated with NAA at 2 mg·L^{-1} and BA at 10 mg·L^{-1} were significantly increased (Table 1).

Figure 1 *C. aromatica* buds in the treatments after two weeks: (A) Control, (B) BA 2–15 mg·L⁻¹, (C) NAA 1–2.5 mg·L⁻¹, (D) GA_3 5–20 mg·L⁻¹, (E) Ethephon 5–20 mg·L⁻¹. For plant growth regulators, from left to right are: (B) BA 2, 5, 10, and 15 mg·L⁻¹; (C) NAA 1, 1.5, 2, and 2.5 mg·L⁻¹; (D) GA_3 5, 10, 15, and 20 mg·L⁻¹; (E) Ethephon 5, 10, 15, and 20 mg·L⁻¹. The scale bars correspond to 1 cm.

Table 1 Changes in diameter, fresh weight, and dry weight of *C. aromatica* rhizomes after two weeks of treatment

Treatments	Diameter (mm)	Fresh weight (mg)	Dry weight (mg)
Control	1.49 ± 0.02^{k}	18.05 ± 0.08^{k}	3.15 ± 0.04^{k}
NAA 1.0 mg·L⁻¹	1.94 ± 0.13^{fg}	18.50 ± 0.23^{hj}	6.60 ± 0.43^{j}
NAA 1.5 mg·L⁻¹	2.31 ± 0.04^{e}	25.50 ± 0.45^{e}	11.57 ± 0.17^{e}
NAA 2.0 mg·L⁻¹	3.03 ± 0.09^{a}	29.13 ± 0.80^{c}	13.43 ± 0.35^{d}
NAA 2.5 mg·L⁻¹	2.34 ± 0.03^{e}	21.00 ± 1.11^{g}	8.40 ± 0.37^{h}
BA 2.0 mg·L⁻¹	2.35 ± 0.03^{e}	21.60 ± 0.30^{fg}	10.13 ± 0.29^{fg}
BA 5.0 mg·L⁻¹	3.00 ± 0.05^{bc}	22.50 ± 0.17^{f}	15.17 ± 0.38^{d}
BA 10.0 mg·L⁻¹	3.19 ± 0.09^{a}	34.2 ± 0.52^{a}	24.76 ± 0.35^{a}
BA 15.0 mg·L⁻¹	2.83 ± 0.06^{b}	29.10 ± 0.60^{c}	19.90 ± 0.11^{b}
GA_3 5.0 mg·L⁻¹	1.71 ± 0.07^{i}	17.23 ± 0.12^{k}	10.85 ± 0.33^{ef}
GA_3 10.0 mg·L⁻¹	2.04 ± 0.03^{fg}	22.53 ± 0.14^{f}	12.63 ± 0.26^{d}
GA_3 15.0 mg·L⁻¹	2.62 ± 0.06^{d}	27.03 ± 0.48^{d}	16.40 ± 0.43^{c}
GA_3 20.0 mg·L⁻¹	2.85 ± 0.01^{b}	30.56 ± 0.31^{b}	25.17 ± 0.13^{a}
Ethephon 5.0 mg·L⁻¹	1.75 ± 0.03^{hi}	17.63 ± 0.27^{k}	7.66 ± 0.28^{h}
Ethephon 10.0 mg·L⁻¹	2.06 ± 0.02^{f}	20.67 ± 0.29^{g}	11.19 ± 0.24^{e}
Ethephon 15.0 mg·L⁻¹	1.91 ± 0.01^{fgh}	19.20 ± 0.21^{h}	9.43 ± 0.17^{g}
Ethephon 20.0 mg·L⁻¹	1.85 ± 0.03^{hi}	17.67 ± 0.20^{k}	8.44 ± 0.27^{h}

NOTE: Means followed by different letters in a column, differ significantly according to the LSD test at the 0.05 probability level.

3.2 Effects of PGRs on early growth stages of C. aromatica rhizomes

There was no significant difference in the number of vascular bundles formed in the control, 20 mg·L⁻¹ GA_3 and 10 mg·L⁻¹ Ethephon treatments. The 10 mg·L⁻¹ BA treatment showed a significant increase in the number of vascular bundles compared with the control and all other treatments. However, it also exhibited the lowest number of PTM cells, significantly lower than the 20 mg·L⁻¹ GA_3 treatment (Table 2, Figure 2).

Treatment with 10 mg·L⁻¹ BA had the highest essential oil content among all treatments. The essential oil content in the control did not differ significantly from the GA_3 20 mg·L⁻¹ and Ethephon 10 mg·L⁻¹ treatments. The treatments had higher sugar content than the control except for the treatment with 20 mg·L⁻¹ GA_3, while the starch content in all PGR treatments was significantly lower than the control, and no treatment showed an increase relative to the control (Table 3).

Respiration intensity with 2 mg·L⁻¹ NAA or 10 mg·L⁻¹ BA treatment was the highest among all treatments (Table 3). Auxin, zeatin, and gibberellin activity were all increased, while abscisic acid (ABA) activity was decreased in both NAA 2 mg·L⁻¹ and BA 10 mg·L⁻¹ treatments compared with controls. In particular, auxin activity reached its highest level when treated with NAA at a concentration of 2 mg·L⁻¹, whereas the activities of zeatin and gibberellin were most strongly enhanced in the treatment supplemented with BA. In contrast, the activity of endogenous hormones in the treatments with GA_3 at 20 mg·L⁻¹ or Ethephon at 10 mg·L⁻¹ did not show any significant difference compared with the control (Table 4).

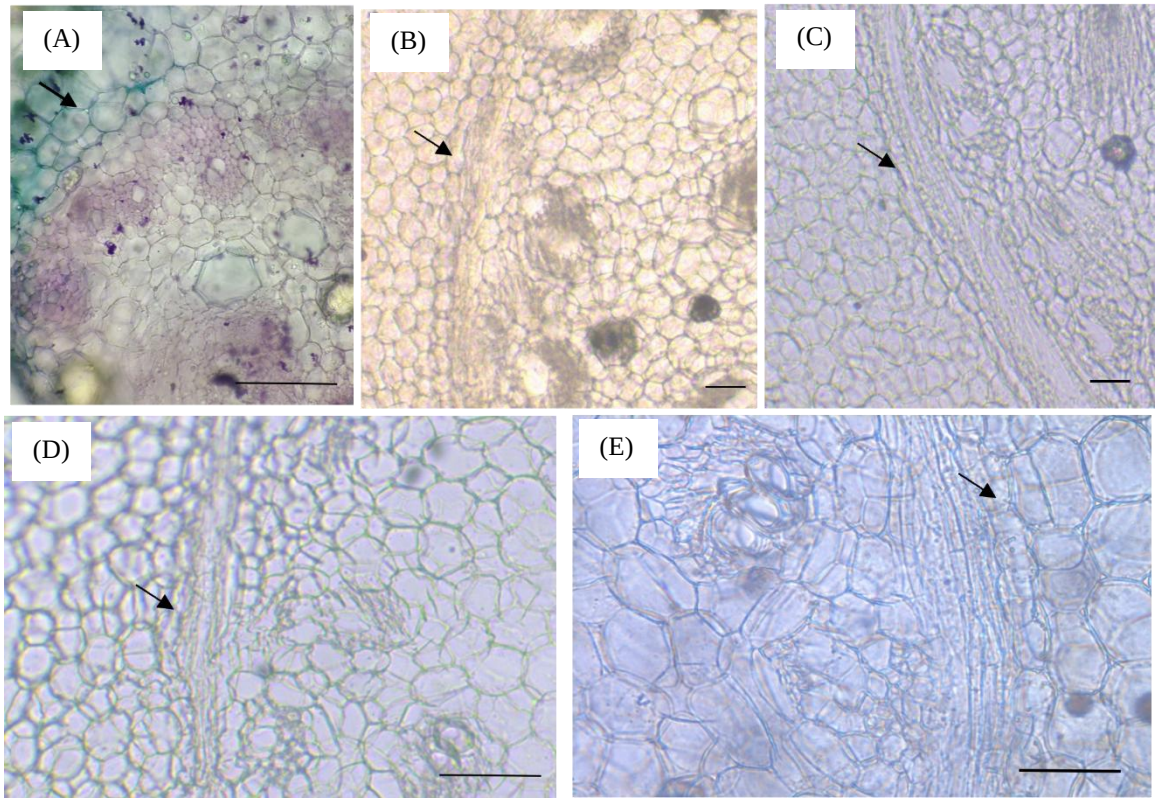

Figure 2 Cross-sections of the edematous part of Curcuma aromatica rhizomes show PTM layers (arrow) and new vascular bundles from PTM layers in the two weeks following treatments: Control (A), 10 mg·L⁻¹ BA (B), 2 mg·L⁻¹ NAA (C), 20 mg·L⁻¹ GA_3 (D), 10 mg·L⁻¹ Ethephon (E). The scale bars correspond to 50 µm.

Table 2 Changes in the number of vascular bundles and PTM cells, of *C. aromatica* rhizomes two weeks after treatment with PGRs

Treatments	PTM cells	Number of vascular bundles
Control	5.67 ± 0.58^{b}	50.67 ± 2.53^{c}
2 mg·L⁻¹ NAA	9.00 ± 0.17^{a}	66.67 ± 0.33^{b}
10 mg·L⁻¹ BA	3.67 ± 0.03^{d}	76.00 ± 1.31^{a}
20 mg.L^{-1} GA_3	2.33 ± 0.07^{c}	49.33 ± 2.53^{c}
10 mg·L⁻¹ Ethephon	6.03 ± 0.03^{b}	50.67 ± 2.53^{c}

Means followed by different letters in a column, differ significantly according to the LSD test at the 0.05 probability level.

Table 3 Changes in sugar content, starch content, essential oil content and respiration intensity (RI) of *C. aromatica* rhizomes two weeks after treatment with PGRs

Treatment	Sugar content (mg.g^{-1} DW)	Starch content (mg.g^{-1} FW)	Essential oil content (mg.100 g^{-1} FW)	RI (µmol O_2 g^{-1} FW h^{-1})
Control	175.57 ± 5.59^{d}	42.17 ± 1.04^{a}	13.33 ± 1.76^{c}	0.41 ± 0.05^{b}
2 mg·L^{-1} NAA	273.54 ± 7.89^{b}	32.31 ± 4.24^{b}	32.33 ± 1.45^{b}	0.48 ± 0.10^{a}
10 mg·L^{-1} BA	316.02 ± 5.10^{a}	33.01 ± 2.96^{b}	40.33 ± 3.93^{a}	0.50 ± 0.05^{a}
20 mg·L^{-1} GA_3	176.48 ± 0.48^{d}	22.48 ± 2.67^{a}	10.67 ± 1.86^{c}	0.38 ± 0.09^{b}
10 mg·L^{-1} Ethephon	196.48 ± 6.65^{c}	25.34 ± 2.02^{b}	11.67 ± 4.05^{c}	0.40 ± 0.04^{b}

Means followed by different letters in a column, differ significantly according to the LSD test at the 0.05 probability level.

Table 4 Changes in endogenous phytohormone activity of *C. aromatica* rhizomes two weeks after treatment with exogenous PGRs

Treatment	Endogenous phytohormone activity (mg·L^{-1})			
	Auxin	Zeatin	Gibberelin	ABA
Control	1.38 ± 0.05^{b}	1.36 ± 0.07^{c}	0.09 ± 0.02^{c}	0.49 ± 0.02^{a}
2 mg·L^{-1} NAA	2.45 ± 0.04^{a}	1.52 ± 0.03^{b}	0.52 ± 0.04^{b}	0.35 ± 0.03^{b}
10 mg·L^{-1} BA	1.36 ± 0.07^{b}	1.69 ± 0.02^{a}	0.72 ± 0.02^{a}	0.34 ± 0.05^{b}
20 mg·L^{-1} GA_3	1.20 ± 0.05^{b}	1.32 ± 0.07^{c}	0.14 ± 0.03^{c}	0.43 ± 0.05^{a}
10 mg·L^{-1}Ethephon	1.40 ± 0.02^{b}	1.29 ± 0.05^{c}	0.10 ± 0.01^{c}	0.41 ± 0.02^{a}

Means followed by different letters in a column, differ significantly according to the LSD test at the 0.05 probability level.

4. Discussion

4.1 Effects of PGRs on early growth stage of rhizomes

All treatments significantly affected rhizome diameter with the application of both NAA 2 mg·L^{-1} and BA 10 mg·L^{-1}. The more vascular bundles produced, the larger the rhizome diameter (Figure 2; Table 2). Thus, the number of vascular bundles greatly contributed to the increase in diameter of *C. aromatica*. In addition, vascular bundles are formed by the action of PTM, when dividing in a straight line, PTM will form many tangential cell layers and if the division is disordered, it will differentiate into vascular bundles (Figure 2). Application of NAA 2 mg·L^{-1} at the initiation stage of white turmeric increased endogenous auxin and zeatin activity, but auxin activity was greater than that of zeatin compared with the control. This is similar to findings in potatoes, where auxin induces tuberization because there is an increase in auxin levels in the stolon tips prior to tuber swelling [17–19]. In white turmeric, perhaps auxin promotes PTM division tangentially to produce more aligned cell layers than vascularization at the initial stage (Figure 3; Table 2), suggesting a major role of auxin in inducing cell division and cell elongation in cambial development, producing procambium in the shoot apex [11], as well as maintaining cambial activity in white turmeric. Otherwise, cytokinins stimulate disorganized division to form vascular bundles, thereby significantly increasing the diameter of the white turmeric bud (Figure 3; Table 2). Indeed, when applying BA 10 mg·L^{-1} at the early stage of bud development, the activity of zeatin was much higher than that of auxin compared with the control, and the PTM activity was strong with disordered cell division, as shown by the gradual reduction of PTM cells, resulting in the strong differentiation of the surrounding cells (Figure 3, Table 2 and 5). Increased cytokinin stimulates the activity of the primary thickening meristem and shortens the time from resting bud to the seedling stage in white turmeric, so vascular bundle formation is stronger, tuber diameter and tuber fresh weight increase sharply, and shoots grow rapidly, with leaf expansion occurring earlier in plants treated with 2 mg·L^{-1} NAA compared with all others (Figure 1 and 2). These results are in agreement with research by Zierer (2021) [20] and Sisay *et al*. (2020) [21], showing that cytokinins stimulate cell division and control morphogenesis, and break apical dominance and promote tip meristem proliferation. Cytokinin may promote tuber formation by increasing glycolysis and ATP synthesis activity [22–24] and stimulating cambial activity similar to that in dicotyledonous plants [11].

The effects of exogenous ethylene and gibberellin are not yet clear in the treatments on white turmeric shoots, similar to studies on ginger, althought they are used as sprouting agents and are involved in stolon elongation and inhibition of tuber formation in potatoes. Foliar application of ethephon promotes tuber formation but reduces the weight of marketable tubers [25]. However, the increase in endogenous GA_3 activity with NAA at 2 mg·L^{-1} or BA at 10 mg·L^{-1} treatment increased shoot length, fresh weight, dry weight, and essential oil content of white turmeric (Fig. 2, Table 3). Therefore, GA_3 is likely involved in tuber metabolism, as discussed below.

4.2 Effects of PGRs on tuber metabolism

In *C. aromatica*, there is a very early accumulation of dry matter, which accompanies the growth of the tuber, because the rhizome diameter and fresh weight increase, the dry weight, the content of starch, and essential oils also increase very soon. Interestingly, the treatment with 2 mg·L^{-1} NAA or 10 mg·L^{-1} BA at the bud initiation stage of white turmeric resulted in both increased growth and essential oil accumulation, with the highest values

observed in the BA 10 mg·L^{-1} treatment. This confirmed that cytokinin and auxin, in addition to stimulating the activity of PTM, also play a role in increasing the accumulation of essential oils in white turmeric. Cytokinin mobilizes sugar from the mother rhizome to the recipient cell in the seedling, whereas NAA inhibits the activity of the enzyme β-amylase to reduce the formation of starch [26]. This cumulative increase could not have been without the role of gibberellin, although when GA_3 was treated at 20 mg·L^{-1}, the essential oil content, dry matter, and gibberellin activity were not different from the control. As endogenous gibberellin activity increased, the content of dry matter, sugar, starch, and essential oil also increased when treated with 2 mg·L^{-1} NAA or 10 mg·L^{-1} BA. Moreover, when starch content decreased, sugar and essential oil content increased (Table 3), indicating that gibberellin acts as a signal to help hydrolyze starch into sugar for plant growth [27], and this is then used for essential oil biosynthesis, which in turn may lead to increased movement of sugars into the essential oil biosynthetic pathway.

5. Conclusion

In vitro treatment of isolated buds with NAA (2 mg·L^{-1}), BA (10 mg·L^{-1}), GA_3 (20 mg·L^{-1}), and Ethephon (10 mg·L^{-1}) significantly increased bud fresh weight, dry weight, and diameter. Specifically, treatment with 10 mg·L^{-1} BA enhanced endogenous levels of auxin, zeatin, and gibberellin, and led to notable increases in fresh and dry weight, the number of vascular bundles, rhizome diameter, sugar content, and essential oil concentration.

We recommend further investigation into the role of plant hormones in regulating essential oil accumulation in white turmeric, particularly during the later stages of growth.

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7. Author Contributions

Tran Thi Thanh Hien: Conceptualization, Methodology, Supervision, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.; Vinh Nguyen Hong Buu: Investigation, Validation, Resources.

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