Increased yield and essential oils content in rhizomes of white turmeric (*Curcuma aromatica* Salisb.) by plant growth regulators

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Abstract
White turmeric is a rhizome plant that contains essential oils and many secondary metabolites having medicinal effects. In order to improve rhizome yield and essential oil content, this study investigated the response of plant growth regulators to the growth stages of white turmeric plants grown from mother tubers in an experimental garden. After 2 weeks of treatment, the rhizomes were measured for fresh and dry weight, rhizome length, sugar and starch content. Rhizome yield, number of rhizomes per plant and essential oil content were measured after 24 weeks of treatment. The results showed that fresh and dry weight as well as sugar content in white turmeric rhizomes at the resting shoot stage increased after 2 weeks of treatment with concentrations of 2 mg/L NAA and 10 mg/L BA, while this occurred at the elongated shoot stage when treated with BA 10 mg/L and at the seedling stage when treated with 20 mg/L GA_3 or 10 mg/L BA to compare with the control and other treatments.

However, at the seedling stage, the rhizome length when treated with 20 mg/L GA_3 was longer, so the fresh weight of the rhizome was more when treated with 10 mg/L BA. 24 weeks after the combined treatment of plant growth regulators (treated with 10 mg/L BA at week 0, treated with 10 mg/L at week 2 and treated with 20 mg/L GA_3 at week 4), the rhizome yield per plant, the number of rhizomes and the essential oil content increased significantly compared to the other treatments. The effects of plant growth regulators on the yield and accumulation of white turmeric rhizomes are also discussed.

Keywords: Essential oil content, plant growth regulators, rhizome yield, white turmeric.

Introduction

White turmeric (*Curcuma aromatica* Salisb.) is used as an aromatic cosmetic and also as a medicine for medicinal purposes because the rhizome of white turmeric consists of alkaloids, curcuminoids, flavonoids, tannins and essential oils which are believed to be the source of medicinal properties[2,3,19]. Tuber yield depends on stem, rhizome and tuber development including storage organ formation, expansion and maturation. The formation of storage organs is a highly complex process and is controlled by many plant growth regulators (PGR) such as auxin, cytokinin, gibberellin and ethylene. Inputs from hormonal signaling are drivers of organogenesis[4,11,17]. Auxin plays an important role in potato tuberization, especially in the process of tuber initiation and growth[11].

Cytokinins play a major role in cell division, vascular cambial activity and secondary growth in crop plants. They have an important function in tuberization, induce tuber production *in vitro* and improve both tuber yield and quality[12,15,22]. Gibberellins were able to induce cell expansion at the onset of tuberization, which increases yam tuber and bulbil yield or the expansion of cells in the later stages of beet root development [10,11]. In white turmeric, the increase in pith size and rhizome diameter due to primary thickening meristem activity occurs very early between the pith and the cortex at the onset of growth (0–6 weeks) with primary vascular bundles formed in the pith[14]. Therefore, studies on the effect of PGR application on PTM activity to increase white turmeric rhizome size in early growth stages should be considered. The aim of the study was to increase the yield and essential oil content of white turmeric by applying plant growth regulators during the first 3 three stages (0, 2 and 4 weeks) of the growth process of white turmeric.

Material and Methods

Explant materials: White turmeric plant at early growth stages: resting shoots, elongated shoots and seedlings from mother rhizome in Center for Research on Plant and Animal Breeding Đak Lak were grown in the experimental garden of the University of Sciences.

Study on effects of single PGRs application on early growth stages of rhizome arising from mother rhizomes in the experimental garden after 2 weeks: White turmeric tubers (mother rhizome) were grown in a pot filled with 2 kilos of potting mixture (rice husk, cow dung and coconut fiber in the ratio 1:1:1) and irrigated once daily at 10 a.m. Plant growth regulators in different concentrations: NAA 2 mg/L, BA 10 mg/L and GA_3 20 mg/L are treated at the early growth stages of tubers. There are 4 treatments: control (treated with water), treated with NAA 2 mg/L, treated with BA 10 mg/L and treated with GA_3 20 mg/L. After 2 weeks of treatment, the changes in fresh weight, dry weight, rhizome diameter, sugar and starch content of primary finger (first-order branch) rhizomes arising from mother rhizome were analyzed.

Table 1
Treatments combined treatment of PGRs on early growth stages of White turmeric rhizome

Treatments	Treatment method
Control	Water
NAA (w 0)	2 mg/L NAA at week 0
BA (w 0)	10 mg/L BA at week 0
BA (w 2)	10 mg/L BA at week 2
GA_3 (w 4)	20 mg/L GA_3 at week 4
Combination 1	2 mg/L NAA at week 0, 10 mg/L BA at week 2, 20 mg/L GA_3 at week 4
Combination 2	10 mg/L BA at week 0, 10 mg/L BA at week 2, 20 mg/L GA_3 at week 4

Study on effects of combined treatment of PGRs on early growth stages of rhizome arising from mother rhizomes in the experimental garden after 24 weeks: Plant growth regulators are most appropriate for each growth stage of treated white turmeric, either alone on every stage or combined in growth stages on white turmeric rhizomes. Treatment includes 7 treatments (Table 1). After 24 weeks of treatment, the rhizome yield per plant, number of rhizomes and rhizome essential oil content were determined.

Measurement of rhizome diameter, fresh weight and dry weight: The rhizome diameter was measured by a caliper and clamped to the largest edematous area of the tuber, then the tubers were weighed immediately to determine the fresh weight and were dried at 120 °C for one hour and at 80 °C until the weight remained unchanged (approximately 72 hours) to determine the dry weight.

Measurement of total sugar and starch content in rhizome: One gram of fresh rhizomes from various treatments was crushed and the total sugar content was extracted with ethanol (four times: three times with 70% ethanol and the last with 90% ethanol). The remaining starchy residue containing starch was dried and hydrolyzed with perchloric acid. The extract was color-reacted with 5% phenol in concentrated sulfuric acid and the optical density was measured at 490 nm using a standard curve with the sucrose solution to determine the sucrose content and glucose solution by multiplying the conversion factor by 0.9 to determine glucose content[7].

Measurement of essential oil content in rhizome: The essential oil content of 1000 grams of fresh rhizomes was determined by steam distillation for 4 hours using a Clevenger apparatus and the resulting essential oil was collected and dehydrated using anhydrous sodium sulfate [6,10].

Statistical analysis: The experimental design consisted of randomized blocks with 10 repetitions. The Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows was used for data analysis.

Results

Effects of PGRs on early growth stages of tubers arising from mother rhizome in the experimental garden

Resting shoot stage: The treatment with 2 mg/L NAA and the treatment with 10 mg/L BA had elongated shoots, but the leaves spread in the treatment with 10 mg/L BA. Both the 20 mg/L GA_3 treatment and the control treatment (with water) had closed, pointed buds and no roots (Figure 1A). Treatments with 10 mg/L BA and 2 mg/L NAA had the highest rhizome diameter, fresh weight and dry weight in all treatments. There was no difference in the length of rhizomes between control and treatment. The treatments had a higher sugar content than the control while the starch content was similar (Table 1).

Elongated shoot stage: The 10 mg/L BA treatment produced primary branches (Figure 1B) and resulted in the highest fresh weight, dry weight, rhizome diameter and sugar content compared to all treatments. The rhizome length of the treatment was longer than that of the control but the starch content was not (Table 2).

Seedling stage: All experiments produced primary branches, many leaves, a wide canopy and a tall stem (Figure 1C). When 2 mg/L NAA was applied at the seedling stage, rhizome diameter and length were not different from the control; dry weight and sugar content were higher, but the starch content was lower. Fresh and dry weight in the treatment increased compared to the control while rhizome diameter only increased when treated with 20 mg/L GA_3 or 10 mg/L BA, but the 10 mg/L BA treatment was the highest. However, 20 mg/L GA_3 treatment significantly increased rhizome length and fresh weight, although there was no difference in dry weight between them. In the 20 mg/L GA_3 treatment, the sugar content increased the most compared to the other treatments, while the starch content was the opposite (Table 2).

Effects of PGRS combination treatments on tuber weight and essential oil content after 24 weeks: In all treatments, rhizome yield per plant, number of rhizomes, dry weight and oil essential content were higher than the control, except that the number of rhizomes in the GA_3 treatment (w 4) was not different from the control (Table 3). Combined treatments at the early growth stage significantly increased yield and quality indices, but rhizome yield per plant, dry weight, number of rhizomes and essential oil content in combination 2 (BA 2 mg/L at week 0, BA 10 mg/L at week 2 and GA_3 20 mg/L at week 4) were the highest (Table 3).

Discussion

Applying NAA 2 mg/L or BA 10 mg/L to resting shoots of white turmeric promoted shoot growth, increased rhizome diameter and increased fresh weight and dry weight compared to the control and the GA_3 treatment. According to Nguyen et al[14], in white turmeric plants at the resting shoot stage (week 0), the PTM has 1 to 2 cell layers but is inactive and after 2 weeks, the PTM is strongly active, dividing tangentially into 6 to 7 cell layers and disorderly division into a lot of primary vascular bundles and has increasing rhizome diameter. This showed that NAA 2 mg/L and BA 10 mg/L induced PTM cell division and generation of vascular bundles, thereby increasing the rhizome diameter and fresh weight (Figure 1, table 2).

Table 2
Changes in diameter, length, fresh weight (FW), dry weight (DW), sugar (SC) and starch content (SC) of white turmeric rhizomes 2 weeks after the treatment with PGRs in growth stages

Treatment	Diameter (mm)	Length (mm)	FW (g)	DW (mg/gFW)	SC (mg/g FW)	StC (mg/g FW)
Resting shoot stage						
Control	4.14 ± 0.21^{c}	3.11 ± 0.21^{ab}	0.17 ± 0.03^{c}	27.61 ± 1.47^{c}	14.57 ± 1.59^{d}	11.17 ± 0.04^{a}
NAA	6.33 ± 0.24^{a}	3.22 ± 0.12^{ab}	1.23 ± 0.02^{a}	42.46 ± 1.08^{b}	27.54 ± 1.89^{b}	10.01 ± 0.24^{ab}
BA	6.47 ± 0.47^{a}	3.15 ± 0.17^{ab}	1.27 ± 0.04^{a}	51.00 ± 2.22^{a}	39.02 ± 1.10^{a}	11.01 ± 0.96^{a}
GA_3	4.78 ± 0.27^{bc}	3.34 ±0.18^{a}	0.25 ± 0.01^{b}	26.04 ± 0.59^{c}	17.48 ± 0.48b^{c}	10.48 ± 0.67^{ab}
Elongated shoot stage						
Control	5.75 ± 0.81^{c}	11.55 ± 0.31^{b}	0.29 ± 0.02^{d}	36.03 ± 0.11^{c}	18.67 ± 2.91^{d}	11.80 ± 0.18^{a}
NAA	8.45 ± 0.12^{b}	15.02 ± 0.17^{a}	0.56 ± 0.05^{b}	42.07 ± 0.25^{b}	30.33 ± 3.48^{b}	10.30 ± 0.14^{bc}
BA	13.29 ± 0.56^{a}	15.62 ± 0.27^{a}	1.65 ± 0.06^{a}	60.14 ± 0.13^{a}	45.67 ± 3.45^{a}	9.02 ± 1.95^{c}
GA_3	6.01 ± 0.15^{c}	15.02 ± 0.19^{a}	0.41 ± 0.04^{c}	36.04 ± 0.21^{c}	30.67 ± 1.69^{c}	5.53 ± 1.63^{d}
Seedling stage						
Control	10.57 ± 0.84^{c}	36.01 ± 0.07^{b}	0.69 ± 0.02^{d}	43.34 ± 0.23^{c}	26.14 ± 0.21^{c}	18.50 ± 0.60^{b}
NAA	11.30 ± 0.32^{c}	37.02 ± 0.11^{b}	2.07 ± 0.28^{c}	46.17 ± 0.13^{b}	28.10 ± 0.61^{b}	15.85 ± 0.10^{c}
BA	16.43 ± 0.47^{a}	36.09 ± 0.15^{b}	4.16 ± 0.41^{b}	66.04 ± 0.17^{a}	46.46 ± 0.87^{a}	18.32 ± 0.84^{a}
GA_3	14.51 ± 0.56^{b}	42.02 ± 0.17^{a}	4.59 ± 0.46^{a}	66.16 ± 0.26^{a}	46.96 ± 0.99^{a}	13.12 ± 1.03^{d}

The means followed by the same letter in each column for each stage are not significantly different at p =0.05

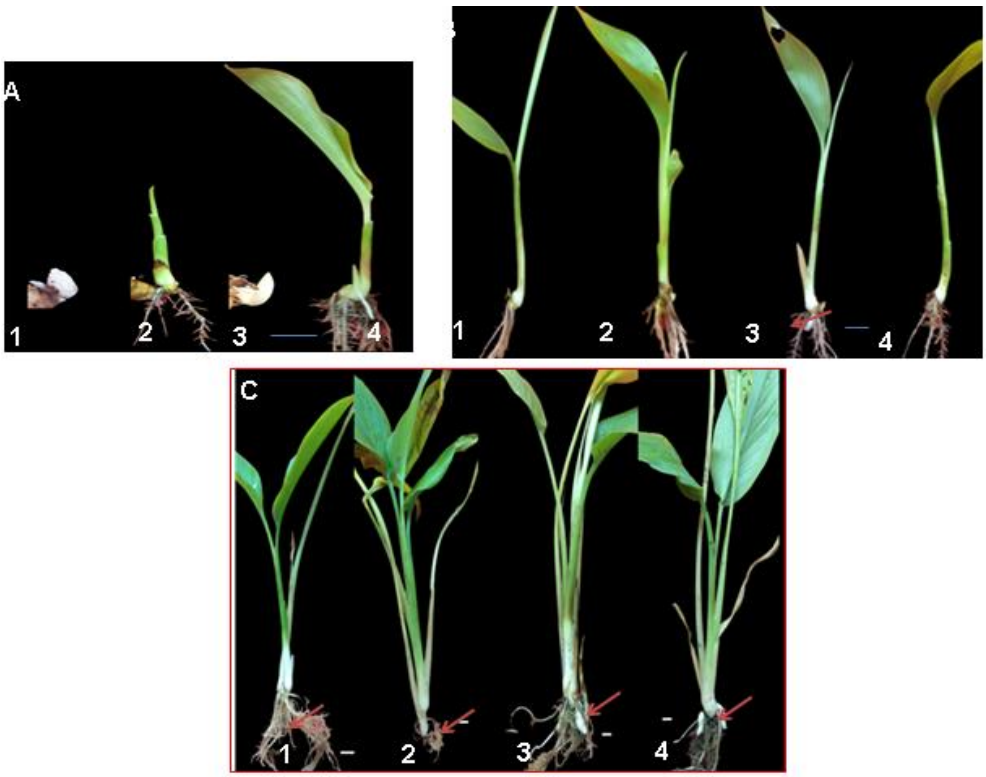

Figure 1: White turmeric after 2 weeks of PGRs application in growth stages: (A) resting shoot, (B) elongated shoot and (C) seedling stage. Left to right: (1) Control, (2) 2 mg/L NAA, (3) 20 mg/L GA_3 and (4) 10 mg/L BA. The bars correspond respectively to 1 cm.

Table 3
Changes in rhizome yield per plant (g), dry weight (mg/g FW), number of rhizomes and essential oil content (OEC) of white turmeric tubers 24 weeks after the treatment with PGRs combination

Treatment	Rhizome yield/plant (g)	Dry weight (mg/g FW)	Number of rhizomes	OEC (%)
Control	215.15 ± 1.60 ^{e}	4.16 ± 0.05 ^{g}	7.00 ± 0.32 ^{e}	3.84 ± 0.04 ^{f}
NAA (w 0)	230.03 ± 0.32 ^{d}	5.23 ± 0.07 ^{e}	8.40 ± 0.24 ^{d}	4.64 ± 0.03 ^{e}
BA (w 0)	257.43 ± 0.88 ^{c}	5.42 ± 0.04 ^{d}	9.60 ± 0.24 ^{c}	5.20 ± 0.04 ^{d}
BA (w 2)	252.33 ± 0.73 ^{c}	5.94 ± 0.05 ^{c}	11.20 ± 0.20 ^{b}	5.98 ± 0.06 ^{c}
GA (w 4)	252.50 ± 0.96 ^{c}	4.39 ± 0.06 ^{f}	7.40 ± 0.24 ^{e}	3.80 ± 0.03 ^{f}
Combination 1	272.84 ± 0.85 ^{b}	7.14 ± 0.04 ^{b}	13.60 ± 0.24 ^{a}	6.96 ± 0.03 ^{b}
Combination 2	298.04 ± 0.85 ^{a}	7.66 ± 0.03 ^{a}	13.80 ± 0.20 ^{a}	7.40 ± 0.03 ^{a}

Means followed by the same letter in each column are not significantly different at p = 0.05

The effect of auxin gradually decreases in the seedling stage. It is possible that auxin content regulates tuber induction and growth, perhaps by increasing the rate of cell division and expansion in the perimedullary (pith) region of the growing tuber, or that cytokinins stimulate cell division and improve morphology, break apical dominance and promote proliferation of the apical meristem[8,11]. The effect of BA on PTM was especially stronger than that of NAA when white turmeric was treated for 2 weeks during the elongated shoot stage. PTM activated at week 14 suggests that the role of BA not only acts in cambial division in white turmeric but also stimulates vascular differentiation of white turmeric in dicotyledonous plants[21], thereby increasing rhizome diameter (Table 2). This effect of BA on tuber diameter persisted at week 4 (seedling stage), but PTM activity decreased as the inner cell layer of the cortex became endodermis.

PTM acts mainly in a tangential direction, creating multiple aligned cell layers, after which cell layers divide in a disorderly manner to form vascular bundles until the endodermis appears and gradually decreases until the endodermis cell wall is filled with lignin in week 14. So the effect of NAA on rhizome diameter is mainly effective at the rest of the of the shoot stage, then gradually decreases until the seedling stage.

Treatment with 20 mg/L GA_3 at the early stage (0 or 2 weeks) did not increase the rhizome diameter of white turmeric but increased the rhizome diameter and rhizome length at the seedling stage. This increased the fresh weight of the rhizomes (Table 2). This is consistent with the findings on potato plants, in which gibberellin prevented tuber formation but stimulated stolon growth similar to the results of Chien et al[5] was studied gibberellin stimulated ginger rhizome elongation. Therefore, GA_3 stimulated the growth of white turmeric rhizomes and the rhizome yield of plants increased, but the number of rhizomes was not different from the control after 24 weeks of treatment (Table 2).

White turmeric has accumulated dry matter from the resting shoot stage. This accumulation occurs as the rhizome grows and as the rhizome diameter and fresh weight of the rhizome increase, so does the dry weight. Treatments with 10 mg/L BA and 2 mg/L NAA increased dry matter compared to the control whereas treatment with 20 mg/L GA_3 only increased dry matter at the seedling stage. In particular, the 10 mg/L BA treatment had the highest dry weight compared to the other treatments at all stages. This shows that cytokinins have the effect of mobilizing sugars from the mother tuber to the uptake cells of the seedlings, resulting in an increase in the dry matter of white turmeric during the early growth stage[20,22].

Therefore, the sugar content in the BA treatment was always highest during the resting and elongating shoot stages compared to the seedling stage and at the seedling stage, the sugar content remained unchanged compared to the control but decreased when compared to other treatments. At the seedling stage, the dry matter of the rhizomes accumulated a lot and when the tubers were 24 weeks old, the essential oil content in the BA treatment was still higher than the other treatments (Table 3).

Similar to NAA treatment, starch content continued to decrease from the resting bud stage to the seedling stage, possibly because NAA inhibited the activity of the enzyme β-amylase, thereby reducing starch formation as in unripe bananas[16] and thus this may lead to an increase in sugar displacement along the essential oil[22] biosynthetic pathway, thereby increasing the essential oil content compared to the control. In particular, at the treatment level of 20 mg/L GA_3, rhizome diameter and essential oil content were not different from the control after 24 weeks when harvest time approached (Table 3). The starch content in the GA_3 treatments always tended to be lower than the other treatments (Table 3). However, the essential oil content did not increase because gibberellin is a signal to hydrolyze starch into sugars to provide nutrients to plants[18].

Therefore, it is not intended for the biosynthesis of essential oil from white turmeric. In general, cytokinin and auxin, in addition to stimulating PTM activity, also play a role in increasing essential oil accumulation in the rhizomes of white turmeric. In particular, the application of BA significantly increased the number of tubers, as cytokinin affects tuber formation[1,13]. This resulted in increased

rhizome number, rhizome yield, dry weight and essential oil content compared to other PGR treatments (Table 3). Combined treatments at different stages (resting shoot, elongated shoot, seedling stage) with suitable PGRs always improved yield and essential oil content compared to single treatments.

Combination 2 (BA 10 mg/L at the resting shoot stage, BA 10 mg/L at the elongated shoot stage and GA_3 20 mg/L at the seedling stage) was the highest, with the highest rhizome yield per plant. The number of rhizomes, dry weight and essential oil content increased significantly compared to the other (NAA 2 mg/L at week 0, 2 mg/L BA at week 2 and 20 mg/L GA_3 at week 4) after 24 weeks of treatment. This demonstrates the potential to stimulate tuber growth and essential oil accumulation in white turmeric when applied in combination with plant growth regulators at appropriate stages.

Conclusion

Rhizome diameter, fresh weight and dry weight increased when treated with 10 mg/L BA or 2 mg/L NAA at the resting shoot stage and with 10 mg/L BA at the elongated shoot stage and both treatments with 10 mg/L BA or treatment with 20 mg/L GA_3 at the seedling stage. Rhizome length and fresh weight were significantly higher in the GA_3 treatment than in the BA treatment.

Sugar content was always high in the BA treatment and starch content was always low in the GA_3 treatment. After 24 weeks of PGR combination treatment (BA at week 0, BA at week 2 and GA_3 at week 4) at three growth stages, rhizome yield per plant, rhizome number and essential oil content increased significantly when compared to other treatments.

Acknowledgement

This research was funded by Viet Nam National University, Ho Chi Minh City (VNU-HCM) under grant number C2022-18-28.

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(Received 23rd March 2024, accepted 24th May 2024)