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Full Length Article

Effect of Cytokinin and Auxin to Curcuminoid Content of *Curcuma domestica* Explants *In Vitro*

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Abstract

Curcumin in turmeric provides several anticancer effects in various types of cancer. In the pharmaceutical field, tissue culture methods are beneficial because they can produce a secondary metabolite in plant tissue on aseptic artificial media which is useful for treatment. Cytokinins and auxin are generally used as growth regulators *In vitro*. This study was aimed to determine the effect of cytokinin and auxin addition to Murashige-Skoog (MS) media on the content of curcuminoids in turmeric explants. Using a completely randomized design consisting of four treatments, namely, A (control); B (9 mg/L cytokinin + auxin 1 mg/L); C (cytokinin 1 mg/L + auxin 1 mg/L); D (0.1 mg/L + auxin 1 mg/L cytokinin). At first, turmeric plantlets were extracted using 96% ethanol. Then, examination of secondary curcuminoid metabolites was carried out by TLC-densitometry. The results showed that the administration of cytokinin and auxin hormones significantly influenced the levels of curcuminoids with a decrease in the concentration of curcumin and bisdemethoxicurcumin in four different types of media. However, in treatment C it significantly increased the levels of demethoxycurcumin with increasing levels of 0.78 mg/L.

Keywords: *Curcuma domestica*; Curcuminoid; Cytokinin; Auxin; Tissue culture; TLC-densitometry

Introduction

Turmeric (*Curcuma domestica* Val.) is one of the potential medicinal plants in Indonesia. Curcumin in turmeric provides several anticancer effects in various types of cancer such as breast cancer, colon cancer, and pancreatic cancer (Dhillon *et al.* 2008; Bartik *et al.* 2011; Liu and Ho 2018). Curcumin works by suppressing cell proliferation and metastasis and induces cell death. Curcumin also shows a protective effect against tumor formation (Deng *et al.* 2016). Turmeric can be a good therapeutic suggestion for the prevention and cure of cancer. Therefore, it is necessary to produce curcumin efficiently (Pramiastuti *et al.* 2023).

Generative and vegetative propagation of conventional turmeric has several obstacles that are influenced by biotic and abiotic factors which can result in its decreased production (Ramachandran and You 1999). Another obstacle in conventional turmeric cultivation is the need for large quantities of rhizomes as planting material and during the dry season turmeric rhizomes can experience dormancy and are less effective (Ugochukwu *et al.* 2013). The length of time for conventional turmeric cultivation is 10–12 months or 20–24 months for harvesting. Long harvest times can also affect the time to provide planting material (Rahardjo and Rostiana 2005). Therefore, there is a need for a turmeric development technique to produce plants on a large scale with a relatively fast time on limited land and controlled environmental conditions. One alternative that can be used is the *In vitro* propagation of turmeric through tissue culture. The advantages of tissue culture are that it can provide continuous, sterile planting material and can extract large amounts of secondary metabolites (Chaturvedi and Chowdhary 2014).

In the pharmaceutical field, the tissue culture method is advantageous because it can be used for the production of secondary metabolites in plant tissues and also in cells that are maintained in artificial media aseptically which are useful for treatment (Sitorus *et al.* 2013). The growth of a plant is needed by macro- and micronutrient factors, vitamins, growth regulators, and carbon sources (Saad and Elshahed 2012). Both the cytokinin and auxin are very important in tissue culture because they are related to shoot and root formation and overall plant growth (Ngomuo *et al.* 2013).

The effectiveness of these growth regulators is different for the growth and development of different plants (Bharalee *et al.* 2005). Curcuma aromatic propagated on Murashige and Skoog (MS) media with the addition of 5% sucrose, 3 mg/L BAP (6-benzylaminopurine) and 0.5 mg/L

NAA (Naphthalene acetic acid) produced the highest curcumin (Wu *et al.* 2015). So, this study was conducted to determine the effect of cytokinin and auxin administration on MS media Multiplication on curcuminoid content in turmeric (*Curcuma domestica* Val.) explants.

Materials and Methods

Experimental details and treatments

Experimental material: The materials used for planting were Multification MS media (Phytotechlab), sucrose, agar (Phytotechlab), distilled water, growth regulators from cytokinin type such as Benzylaminopurine (BAP) (Phytotechlab); Thidiazuron (TDZ) (Phytotechlab); and Zeatin (Phytotechlab), as well as auxin type i.e., 1-naphthaleneacetic acid (NAA) (Sigma). The source of the plant material used was turmeric explants of the Bogor variety from the Seed Technology Tissue Culture Laboratory, Faculty of Agriculture, Padjadjaran University, Jawa, Indonesia. The materials used for secondary metabolite analysis were 96% ethanol, methanol, chloroform, benzene, GF254 silica gel plates, and curcuminoid as positive control.

Treatments: For the determination of effect of cytokinin and auxin to curcuminoid content of *Curcuma domestica* explant, four sterilized different growth media were prepared i.e. A: Multiplication MS (Control), B: Multiplication MS + 9 mg/L BAP + 1 mg/L NAA, C: Multiplication MS + 1 mg/L TDZ + 1 mg/L NAA and D: Multiplication MS + 0.1 mg/L Zeatin + 1 mg/L NAA with pH 5.6–5.8. Then, *Curcuma domestica* explants that have grown into plantlets were subcultured on those media.

Plant harvesting

The explants were planted in a at Laminar Air Flow by cleaning the base of the stem or bud of turmeric from the roots and the previous media using tweezers and a scalpel so that only the stem and base were left. Then the explants were planted into bottles containing media combinations variation (A, B, C and D media) with tweezers. Bottles were labeled according to the treatment and planting date and stored in a culture room at a temperature of 20 ± 1^0C and 70% humidity with 16 h of light. Harvesting was carried out after explants had grown for 3 months. Assessment of curcuminoid content from explant.

Turmeric explants grown into plantlets were examined for the secondary metabolites by extraction and TLC-densitometry. The subcultured fresh turmeric plantlets were cleaned and dried in an oven at 50^0C for 48 h. Furthermore, it was mashed using a mortar to become powder (Pothitirat and Gritsanapan 2005) and 50 mg of a dried turmeric plantlet was put into a microtube. Then it was extracted with 500 µL of 96% ethanol using a vortex for 3 min at room temperature, centrifuged at 30,000 rpm for 20 min and supernatant was stored (Wahyuni *et al*. 2018). A 5 µL of the supernatant and curcuminoids standard compounds were spotted on GF254 silica gel plates. The silica gel plate was then placed in a thin layer chromatography (TLC) vessel containing the mobile phase of chloroform, benzene, methanol (80:15:5) which was saturated until the eluent reached the upper limit mark (Pothitirat and Gritsanapan 2005). The length of each chromatogram was 8 cm. Then the spots were seen under UV light at 254 nm and 366 nm. The eluted plates were then dried and put into a TLC-scanner and analyzed by densitometry at 420 nm wavelength.

Statistical analysis

The data were statistically analyzed using Duncan Multiple Range Test (DMRT).

Results

Characteristic of *Curcuma domestica* plantlet and the extract of turmeric plantlet

Turmeric plantlet growth in four different treatments A, B, C and D is given Fig. 1. The mean fresh weight of turmeric explant subculture resulted in treatments A, B, C and D was 3.24, 1.34, 2.17, 2.89 g, respectively. Furthermore, the water content of turmeric plantlets in treatments A, B, C and D was obtained 93.59, 90.24, 90.46 and 90.39% respectively. Turmeric plantlet extracts were bright yellow to greenish yellow (Fig. 2).

Analysis results of secondary metabolites of turmeric plantet extract by TLC

The chromatogram pattern showed that using the stationary phase of silica gel plate GF254 and the mobile phase of chloroform, benzene, methanol (80:15:5), there was good separation of compounds of the curcuminoids group i.e., curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Fig. 3). The four treatments A B C and D on the turmeric plants showed the same pattern i.e., all extracts contained curcumin, demethoxycurcumin and bisdemethoxycurcumin.

Curcuminoid content in turmeric plantlet extracts and statistical analysis results

Results showed that three treatments B, C and D indicated reduced curcuminoids content except treatment C, which showed increased demethoxycurcumin, while treatment D had no effect on bisdemethoxycurcumin content (Fig. 4). Those treatments had also lowered the curcuminoids content by more than 50% (Fig. 5). The percentage of curcumin, demethoxycurcumin, bisdemethoxycurcumin to the weight of turmeric plantlets also decreased around 40–70% due to treatments B, C and D (Fig. 6).

Fig. 1: *Curcuma domestica* explant growth for three months on modified Multiplication Murashige-Skoog media
A: *Curcuma domestica* explant on multiplication MS (control)
B: *Curcuma domestica* explant on multiplication MS + cytokinin of 9 mg/L+Auxin of 1 mg/L
C: *Curcuma domestica* explant on multiplication MS + cytokinin of 1 mg/L + Auxin of 1 mg/L
D: *Curcuma domestica* explant on multiplication MS + cytokinin of 0.1 mg/L + Auxin of 1 mg/L

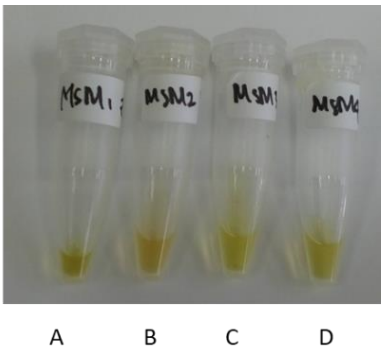

Fig. 2: Result of turmeric plantlet extraction
A: *Curcuma domestica* explant on multiplication MS (control)
B: *Curcuma domestica* explant on multiplication MS + cytokinin of 9 mg/L+Auxin of 1 mg/L
C: *Curcuma domestica* explant on multiplication MS + cytokinin of 1 mg/L + Auxin of 1 mg/L
D: *Curcuma domestica* explant on multiplication MS + cytokinin of 0.1 mg/L + Auxin of 1 mg/L

Discussion

Measurement of the fresh weight of turmeric plantlets showed that plantlets can be affected by the speed at which cells divide, multiply, and grow to become plantlets. In addition, the growth of plantlets within one species can differ depending on the explants' original position and growth conditions. The addition of cytokinins and auxins in treatments B, C and D had reduced the average weight of turmeric plantlets. The addition of cytokines by 9 times in treatment B reduced the growth of turmeric plantlets highly (Fig. 1). The addition of cytokinins to MS medium significantly affected shoot formation but inhibited shoot elongation (Murch and Saxena 2001; Suminar *et al.* 2019). High exogenous cytokinins inhibited root formation (Gaspar and Couman 1987). Besides high exogenous auxin inhibits root formation (Pamungkas *et al.* 2009). This could explain the phenomenon so that at the high cytokinin concentration the total weight of turmeric plantlets decreased by almost 50% (Fig. 5). However, exogenous auxin used in this study was 1 mg/L NAA according to previous results (Suminar *et al.* 2019) which provided optimum root growth in turmeric plantlet.

The water content of these turmeric plantlets showed more than 90% (Fig. 2). It is higher than previously reported i.e., the water content of turmeric is 70–80% (Singh *et al*. 2010). The differences in water content can be caused by many factors such as differences in planting conditions and varieties of turmeric (Hirun *et al*. 2012). The curcuminoids contained in the turmeric plant have been successfully extracted using ethanol. This was performed by the presence of a curcuminoid group i.e., curcumin, demethoxycurcumin, and bisdemethoxycurcumin when the compounds contained in the extract were separated (Fig. 3).

In treatment A, which only used MS30 multiplication media, the highest levels of bisdemethoxycurcumin were found, followed by curcumin and demethoxy (Fig. 6). This is different from the MS0 media treatment on *Curcuma Longa* which produced the highest curcumin content compared to demethoxy and bisdemethoxy curcumin (Pistelli *et al*. 2012). In this study MS was used which contained 30 g/L of sucrose based on previous research which showed that this type of MS media resulted in the best regeneration ability of *Curcuma kwangsis* (Zhang *et al.* 2010).

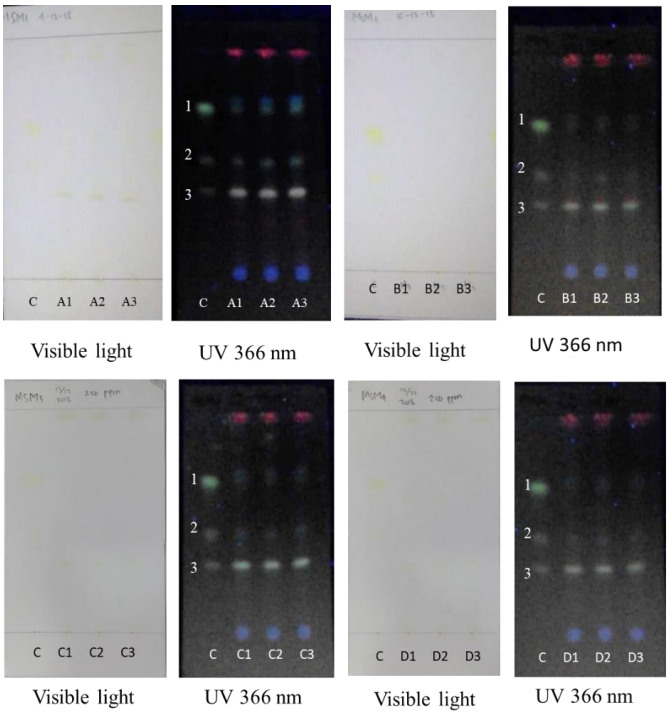

Fig. 3: The chromatogram pattern of TLC results of turmeric plantlet extracts C: curcuminoid standard
1=curcumin, 2= demethoxycurcumin. 3= bisdemethoxycurcumin
A1, A2 and A3= extract from turmeric plantet A
B1, B2 and B3 = extract from turmeric plantet B
C1, C2 and C3 = extract from turmeric plantet C
D1, D2 and D3= extract from turmeric plantet D

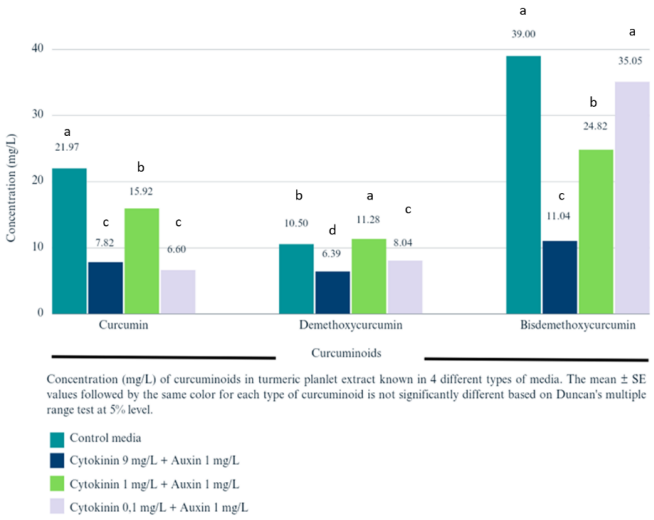

Fig. 4: Curcumin, demethoxycurcumin and bisdemethoxycurcumin content in turmeric plantlet extracts and result of statistical analysis of Duncan's multiple range test at a significant level of 5%. Mean ± standard deviation. Values with different letter differ significantly (P<0.05)

In this study, addition of cytokinin and auxin had a significant effect on decreasing the concentrations of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 4). However, administration of 1 mg/L cytokinin and 1 mg/L auxin (treatment C) significantly increased the concentration of demethoxycurcumin with an increase of 0.78 mg/L compared to treatment A (control) (Fig. 4). Furthermore, demethoxycurcumin has better anti-metastasis effect than curcumin **(**Yodkeeree *et al*. 2009). The result of this research is the same as the addition of Paclobutrazol (PBZ), a growth inhibitor, to turmeric plants with a curcuminoid content below 3% indicating a low

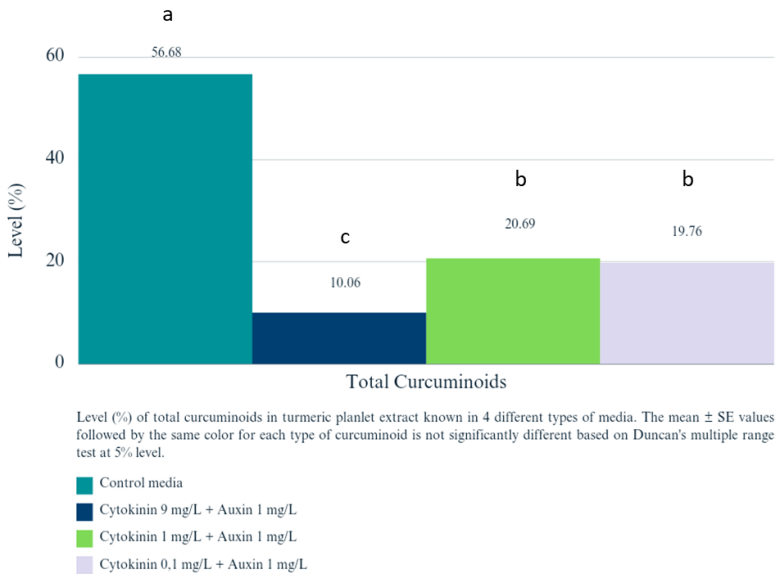

Fig. 5: Curcuminoid content of turmeric plantlet extracts result of statistical analysis of Duncan's multiple range test at a significant level of 5%. Mean ± standard deviation. Values with different letter differ significantly (P<0.05)

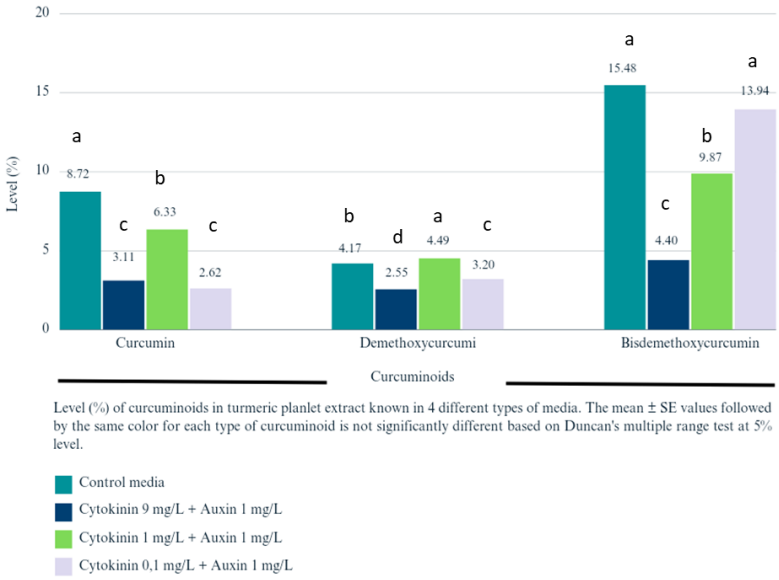

Fig. 6: Weight percentage of curcumin, demethoxycurcumin and bisdemethoxycurcumin to turmeric plantlet weight. Mean ± standard deviation. Values with different letter differ significantly (P<0.05)

curcuminoid content (Chungloo *et al*. 2021). (PBZ) is a growth inhibitor and is commonly used for plants under stress conditions but has the same effect as the addition of auxin and cytokinin on curcuminoid content.

Conclusion

Administration of cytokinin and auxin at different concentrations in MS medium significantly reduced the levels of total curcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric explants. However, addition of cytokinins and auxins at cytokinins and auxin (at 1 mg/L each) significantly increased the demethoxycurcumin level. Variation in the ratio of hormones in turmeric plants has an impact on their curcuminoids content. So that it can be a guide to increase the desired type of curcuminoids.

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Author Contributions

TR, SM, ZZ and ES planned the experiments, TR and SM interpreted the results, ZZ and TR made the write up and ZZ statistically analyzed the data and made illustrations.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

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