

THE EFFECT OF PLANT GROWTH REGULATOR TRIACONTANOL TO THE GROWTH OF CACAO SEEDLINGS (*Theobroma cacao* L.)

Rama R. Sitinjak^{1*)} and Dingse Pandiangan²⁾

¹⁾ Department of Agrotechnology, Prima Indonesia University, Medan
Jl. Sekip Simpang Sikambing, Medan, North Sumatera, Indonesia

²⁾ Department of Biology, Sam Ratulangi University
Jl. Kampus Kleak, Manado, North Sulawesi, Indonesia

^{*)} Corresponding author Phone: +62-812-63757715 E-mail: sitinjakrama@yahoo.co.id

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ABSTRACT

The aim of this study was to evaluate the effect of plant growth regulator triacontanol to the growth of cacao seedlings (*Theobroma cacao* L.). A completely randomized design was applied using non-factorial pattern on the treatment triacontanol growing regulator substance to level: 0.0 ml/L, 0.1 ml/L, 0.5 ml/L, 1.0 ml/L and 2.0 ml/L replicated 5 times. The result shows that the given of triacontanol significantly affected the growth of cacao seedlings at the age of 14 weeks. The best triacontanol concentration was 1.0 ml/L, which was effectively able to increase the growth of cacao seedlings; high seedlings, number of leaves, length of leaves, and diameter of stems respectively reached 26.21%; 3.70%; 42.28%; and 10.42% higher than the control. Plant growth regulator triacontanol was effective to increase the growth of cacao seedlings.

Keywords: triacontanol, seedling, *Theobroma cacao* L.

INTRODUCTION

Cacao (*Theobroma cacao* L.) is of the family of Malvaceae which is tree-shaped originating from South America. In the region of origin, cacao is a small plant at the bottom of the tropical rain forest and is shaded by large trees. In general, the cacao can be divided into two major types, namely Criollo and Forastero. Criollo is high-quality cacao, but in cultivation, it requires intensive treatment. Cacao plants produce seeds that serve as the main ingredient for chocolate. Chocolate is a favorite food, which contains high nutrition. Chocolate is highly nutritious and

required as the ingredient for chocolate candy, ice cream, and various types of food.

This plant is one of the commodities suitable for smallholders, as they can flower and bear fruits throughout the year, which could serve as the source of daily or weekly income for growers. In addition, cacao is also one of non-oil export commodities which fairly has bright prospect for domestic demand that is also stronger along with the development of agro-industry sector. However, if the land is obviously complicated factor, and poor nutrients, especially micro elements, natural hormonal factors, climate and weather, pest and disease factors are not considered, then the level of production and quality will be low. Viewed in term of the quality of the results, people are still unsatisfied with the cacao cultivation, which usually starts from planting to harvesting, especially when the cacao seeds start to germinate. Because cacao seeds have no dormancy period and germination power rapidly declines, then the cacao seeds should be planted (Indonesian Coffee and Cacao Research Institute, 2010).

When the seeds have germinated, they can be planted directly into a polybag. One effort to increase the potency of seeds that have been planted in polybags, is the treatment of plant growth regulator (PGR) is appropriate. Growth regulator is only needed in small amounts to determine the course of physiological processes, growth, differentiation, and development (Davies, 1995). The hormone is only effective within a certain amount. Too high concentrations can inhibit plant growth even lethal, while less optimum concentration may bring to ineffectiveness. Synthetic growth hormone can improve crop yields, both through vegetative propagation and embryogenesis (Gana, 2010).

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One type of plant growth regulator that is able to stimulate plant growth, which is also known as alcohol $C_{30}H_{62}O$ melissyl is triacontanol. These compounds are found in bees wax and cuticle in rice (*Oryza sativa* L.) (Jaybhay *et al.*, 2010). Triacontanol including long chain fatty alcohol actively stimulates plant growth and increases yields when the exogenous with nanomolar concentrations are given on a number of plants (Chen *et al.*, 2002). This growth substances are used to enhance crop yields on millions of hectares, the majority of annual vegetables and agronomic plants as well as several types of forest species (Ries, 1991). Triacontanol given to the seed of some weeds and horticultural crops have an influence on the germination and its morphology (Hoagland, 1980). According to Khandaker *et al.* (2013) that triacontanol sprayed on the leaves can stimulate growth, increase inflorescence, and improve the quality of crops. Samui and Roy (2007) also reported that no phytotoxic effect and no detrimental effect is given to plants and predators, respectively.

The effectiveness of triacontanol on Woody plants have also been successfully investigated although in micropropagation i.e. via organogenesis in plants of apples (*Malus domestica* CV. JTE-E4) and Cherry (*Cerasus fruticosa* CV. Probocskai) (Tantos *et al.*, 2001), and through somatic embryogenesis in plants pine (*Pinus roxburghii*) (Malabadi and Nataraja, 2007), and the coffee plant (*Coffea arabica* L. and *Coffea canephora*) (Giridhar *et al.*, 2004), as well as on the cultures of haploid cells of tobacco (*Nicotiana tabacum*) (Hangarter and Ries, 1978).

Utilization of triacontanol as a growth stimulating substances can also be used to plant cuttings, including Hinerman and Kunkel (1982) succeeded in increasing the growth of root pieces on *Rhododendron hybrid* plant with the treatment of 0.1 mg/L solution of triacontanol. Similarly, Sutedja (1993) has been successful in increasing the growth of short cuttings vanilla (*Vanilla planifolia*) through the increase in leaf area and net assimilation rate at a concentration of 0,1 ml/L triacontanol. When compared with other growth regulator, triacontanol in very small amounts can effectively improve the process of growth and production on various types of plants, including on the plant of *Arachis hypogaea* L. (Verma *et al.*, 2009), *Capsicum annuum* L. plants (Chaudhary *et al.*, 2006), *Solanum melongena* L.

Pusa Purple Cluster (PPC) plants (Sharma, 2006), and the Yellow lupin plants (Borowska and Prusinski, 2011). According to Perveen *et al.* (2014) 10 mM Triacontanol were sprayed on the leaves in order to more effectively reduce the adverse effects of salt stress on growth, yield, and leaf water relations of wheat plants when applied at the vegetative or vegetative + boot growth stages. This growth substance is also effective to normalize metabolic processes on soyben plants treated with salt stress (Krishnar and Kumari, 2008) and chilling stress on the plants of *Ocimum basilicum* L. (Borowski and Blamowski, 2009).

Due to the limited number, it is effective in improving the growth and yield, and no studies implied that the use of triacontanol is to improve the growth and production of cacao. This study was aimed to determine the potential growth of cacao seedlings from application of a growth regulator triacontanol and to determine the optimum levels of triacontanol in enhancing the growth potential of cacao seedlings.

MATERIALS AND METHODS

The study was conducted from March to June 2011 in the gardens of cacao farmers, in Medan, with a height of 435 m above sea level. The study used a completely randomized design (Completely Randomized Design) with five-time replications. The treatment of growth regulator where triacontanol was given was: 0.0 ml/L, 0.1 ml/L, 0.5 ml/L, 1.0 ml/L, and 2.0 ml/L. Each treatment consisted of five cacao seeds, so overall there were 25 samples of cacao seeds. Materials and tools used were local cacao seeds, triacontanol, polybag, medium soil, manure, and sand.

Research procedures:

- a. Preparation and planting: ripe cacao pods were peeled and flesh was cleaned. Germination of seeds was done with the sack. Growing media were used is soil, manure and sand (2:1:1). Growing media were mixed, then put into polybag. Cacao seeds obtained were planted in polybags. After seeds planting, triacontanol was given in accordance with the treatment.
- b. Maintenance: After the treatment with triacontanol, then the pre-filled polybags cacao seeds were arranged in a greenhouse with a distance of 15 x 30 cm.

Furthermore, treatment was done in order to optimally grow cacao seedlings.

- c. Observations: Each treatment was observed for 14 weeks. The parameters measured were seedling height, number of leaves, length of leaves, and stem diameter of cacao seedlings.

The data has been collected and analyzed using ANOVA statistical test. If on the variance the obtained treatment effect F test was significantly different or very real, so to determine the differences in each treatment, further testing using different test real distance Duncan was conducted (Duncan's multiple range test method) with a significance level of 95% (Gomez and Gomez, 1995; Hanafi, 1991).

RESULTS AND DISCUSSION

RESULTS

The results of this study showed that giving of triacantanol on cacao seedlings had a very significant effect on the increase in stem height, number of leaves, leaf length, and stem diameter of cacao seedlings after the age of 14 weeks. The results of different test average on cacao seedling growth parameters through the method of Duncan's multiple range test (DMRT) at each treatment were shown in Table 1. High cacao seedlings could be increased until it reached the average of 26 cm/cacao seedlings (the highest) at a concentration of 1.0 ml/L triacantanol. This treatment was significantly

different from the treatment without plant growth regulator (control). These results indicate that there was an increase by 26.21% when compared with control (the average of height of stems reached 20.6 cm/cacao seedlings). Giving 1.0 ml/L triacantanol was also significantly different from giving higher concentration (2.0 ml/L), where its average stem height obtained was 12.7 cm/seedling.

Similarly, the parameters of leaf length, leaf longest average obtained could reach 16.11 cm/leaves/seedlings when given 1.0 ml/L triacantanol. This treatment was also significantly different from the treatment without plant growth regulator and also to the other treatments. In the parameter of number of leaves, giving a concentration of 1.0 ml/L triacantanol could give the highest number of leaves, ie an average of 11.2 leaves/seedlings. This treatment was not significantly different from that with and without PGR, except for the higher concentration is 2.0 ml/L obtained by the number of leaves averaged only 5.8 leaves/seedlings cacao.

It was similar with the parameters of a stem diameter of cacao seedlings. Giving 1.0 ml/L triacantanol could give the average largest stem diameter of cacao seedlings, ie. 2.12 cm/seedling. This treatment was not significantly different from that with and without PGR other treatments, except for the higher concentration is 2.0 ml/L. The effect of plant growth regulator of triacantanol on the growth of cacao seedlings 14 weeks of age is presented in Figure 1.

Table 1. Difference test average on cacao seedling growth parameters from application of triacantanol by DMRT method

Treatment	High of seedling (cm)	Lenght of leaf (cm)	Amount of leaf	Diameter of seedling (cm)
Ro = 0.0 ml/L	20.60 c	11.74 b	10.80 a	1.92 a
R1 = 0.1 ml/L	24.20 ab	14.49 b	10.00 a	2.02 a
R2 = 0.5 ml/L	22.06 bc	12.52 b	10.20 a	1.86 a
R3 = 1.0 ml/L	26.00 a	16.11 a	11.20 a	2.12 a
R4 = 2.0 ml/L	12.70 d	8.80 c	5.80 b	1.02 b

Remarks: Values followed by the same letters in the same column are not significant at the 5% LSD.



Figure 1. Cacao seedlings 14 weeks old (from left to right: R0 = control with concentration of 0.0 ml/L, R3 = treatment concentration of 1.0 ml/L, and R4 = treatment concentration 2.0 ml/L)

DISCUSSION

Triacantanol is an effective growth regulator that can significantly improve plant growth (Tantos *et al.*, 1999; Tantos *et al.*, 2001; Malabadi and Nataraja, 2007; Perveen *et al.*, 2010; Aziz *et al.*, 2013; Khandaker, 2013; Perveen *et al.*, 2014). These growing regulatory substances are effective on the biosynthesis of secondary metabolites and role plays to set up the process associated with the physiology and biochemistry of plants (Jaybhay *et al.*, 2010).

It was indicated in the study that the concentration treatment of growth regulator triacantanol significantly affected the growth of cacao seedlings including: high seedling, leaf length, leaf number, and stem diameter of cacao seedlings at the age of 14 weeks. It shows that the growth regulator substances can stimulate or inhibit the growth and development of the cacao plant. According to Giridhar *et al.* (2004) during the period of growth, the provision of triacantanol can improve morphogenetic response in plant embryogenesis process. This occurs because growth regulators can increase the speed of cell division to produce more numerous and larger roots and shoots. During the growth and the development in plant tissues, a change occurs in gene expression that result in the synthesis of new proteins and mRNA. Genetic information has led to a variety of physiological and cellular responses that involve changes to the development program of the cells of plant tissues (Chugh and Khurana, 2002). Ries (1991) also argued that the provision of triacantanol can

increase the percentage of formation of L (+) - adenosine in the root tissues of plant seeds. Improvement of L (+) - adenosine may be a result of the action of the enzyme ATPase in membrane vesicles of L (+) - AMP or L (+) - ADP or L (+) - ATP, which plays a role in the process of assimilation in plants. Then He and Loh (2002) states that the change in the total endogenous cytokinin concentration of isopentenyl adenosine subfamily in leaf and root tissues is associated with the onset of bolting induced by triacantanol. Thus, the results of this study indicate that the process of photosynthesis whose activity was enhanced by triacantanol in the leaves were very closely related to the growth of shoots, stems, and leaves, as well as the root growth of the cacao seedlings.

This study also shows that cacao seedlings were given triacantanol with a concentration of 1.0 ml/L, obtaining the highest seedling height (26.21% higher than control) and the highest leaf length (42.28% higher than control) than without triacantanol (control). While giving a higher concentration (2.0 ml/L) could actually inhibit the growth of seedlings (seedling height, leaf length, leaf number, and the stem diameter), and it could even cause cacao to die. This indicates that the concentration of triacantanol 1.0 ml/L was effective to stimulate cell division, especially in the shoot meristem tissue both longitudinally and transversely so that cacao seedlings could grow faster. Certain triacantanol concentration (1.0 ml/L) was more effective to increase the length of the leaves of cacao seedlings, when compared with no PGR triacantanol. The optimum concentration (1.0 ml/L) of triacantanol showed that in order to increase the sensitivity of cells in tissue of cacao seeds, specific PGR concentration was required. Receptors on the cell membrane specifically bind hormone, and different cell response to hormones may be determined by the presence of cell signal transduction molecule specific. Therefore, the growth of seedlings needed optimum concentration, which can increase the sensitivity of cells to actively initiate the formation of tissue or activate specific genes to induce the growth of cacao seedlings. The seed cells are more sensitive in receiving stimulation of a growth regulator to affect the physiological processes of seed, so it can improve the root system, improve absorption of nutrients, and increase the amount of chlorophyll, as well as increase the assimilation process

(Chen *et al.*, 2002). Triacantanol application can also cause an increase in the intensity of photosynthesis in the leaves (Borowski and Blamowski, 2009), the increase in CO₂ assimilation rate, stomatal conductance, and water efficiency (Aziz *et al.*, 2013), increased transpiration rate, ratio of chlorophyll a / b, electron transport rate, and the content of K⁺ in the roots (Shahbaza *et al.*, 2013), an increase in nucleic acids, sugar solution, and proteins (Krishnan and Kumari, 2008). Additionally, triacantanol can encourage the uptake of Fe²⁺ ions (Kusumo, 1990). Fe²⁺ ions are needed by plants in the formation of the precursor of chlorophyll in chloroplasts and as electron carriers in photosynthesis. Photosynthesis rate can be regulated by triacantanol by activating secondary messengers that play a role in increasing the enzymatic activity of the plant, so the plant can increase vegetative growth (Chen *et al.*, 2002). According to Hinerman and Kunkel (1982) triacantanol directly activates the genes that control the process of photosynthesis. These genes in turn activate enzymes that control the chemical process of photosynthesis. Therefore, it is likely that triacantanol given the optimum concentration can more effectively increase the activity of enzymes to control the process of photosynthesis that occurs in the cells of the leaves of cacao seedlings. As with any ANT gene (Aintegumenata) which is the regulatory genes of Arabidopsis. ANT regulates cell proliferation and organ growth by maintaining the meristematic competence of cells during organogenesis (Mizukami and Fischer, 2000). Thus, it is possible that triacantanol given on the optimum concentration will promote the expression of genes, so that it will activate a particular gene to control the process of organogenesis to initiate a shoot and root of the growth of cacao seedlings. Naeem *et al.* (2012) also reported that triacantanol possible role in regulating growth and metabolism of plants lies in the regulation of gene expression. According to Hangarter and Ries (1978) triacantanol potentially increases growth due to the increase in the number of cells in the plant. The activated genes in gene expression are due to the provision of triacantanol, the potential possibility to control the amount of cells from plant organs and organ size of the overall development of shoots and roots of the cacao seedlings. Pal *et al.* (2009) also reported that in addition to the results, the application of triacantanol had a positive effect

on leaf area index at the plant. The presence of leaves on the seedlings are a good influence on the formation of roots, because the leaves can provide food materials through photosynthesis and shoots as a source of endogenous auxin. Tantos *et al.* (2001) and Verma *et al.* (2011) also argued that the provision of triacantanol could increase the number of roots per plant, and this effect could be improved when combined with auxin indole-3-butyric acid (IBA).

This study also showed that giving of triacantanol could increase the number of leaves and stem diameter of the cacao seedlings. The number of leaves and the highest stem diameter of cacao seeds were obtained on treatment of 1.0 ml/L triacantanol. Although this treatment was not significantly different from other treatments, except when compared with the higher concentration (2.0 ml/L), but this showed that the growth regulating substances in small amounts could stimulate the growth of plant cells and tissues, but in high amounts, it could hamper, or could even kill plants. Basically, the addition of a trunk diameter is influenced by endogenous auxin produced by the leaves or shoots that actively grow. Auxin is synthesized in apical buds and young leaves, will be transported to the stem to regulate cell division in the vascular cambium, which is involved in secondary growth, thus causing stem elongation and enlargement. Auxin also initiates the differentiation of cells in the leaves and arranges the extension and enlargement of young leaves (Fosket, 1994). Cytokinin and auxin interact in controlling the formation of the shoot apical meristem and the root apical meristem in the early embryogenesis of plants (Su *et al.*, 2011). According to Ruzicka *et al.* (2007) root growth was triggered by the activity of post-embryonic cells in the root meristem controlled by the coordinated action of several phytohormones. Hormone cytokinin stimulates the differentiation of cells in the roots of plants with the emphasis on auxin transport and auxin response on the border between the root meristem and elongation zone (Chapman and Estelle, 2009). Similarly, stimulating plant stem extension due to the cooperation of several types of hormones involves auxin and cytokinin. Auxin plays a role in stimulating the growth of the cell expansion (Yang *et al.*, 1996), while cytokines stimulate the growth, where there is the possibility potentially limiting of cell expansion, thereby accelerating the balance between of the cell ex-

pansion and cell division needed for maximum growth (Hagen *et al.*, 1975). Giving triacantanol the optimum concentration of events will effectively stimulate signaling pathways in cells, and effectively balance the ratio hormone endogenous to exogenous hormones working together in a coordinated fashion to control the growth of cacao seedlings through the process of cell division, cell elongation, and set the balance so as to produce the maximum growth of the seedling height, stem diameter, number of leaves, and leaf width. According to Jimenez (2001); Chaudhury and Qu (2000), in order to determine the induction and expression of the growth and development of young plants, a certain ratio of endogenous and exogenous hormones is required. The effectiveness of exogenous growth regulators depends on the concentration of endogenous hormones (Bhaskaran and Smith, 1990). Then according to Cavusoglu *et al.* (2008) triacantanol is very effective in trunk diameter, which is able to increase the width of the vascular vessels, xylem width, and the width of the phloem.

Therefore triacantanol can also stimulate cell division, this indicates the possibility of the existence of the right combination of triacantanol exogenous to auxin hormone in the growth of cacao seedlings. Therefore, giving 1.0 ml/L triacantanol was able to increase the growth of cacao seedlings at the age of 14 weeks. This concentration was effective to stimulate cell division, cell elongation, and formation of new cells so that the apical buds produced new leaves and plant height at the same time increasing stem diameter, and the increase in the number of leaves and leaf length, resulting in the increase of photosynthesis. The results were then used for the growth of cacao seedlings. Increasing the number of leaves and leaf length is in line with the increasing frequency of chloroplasts, meaning that higher photosynthetic production results in faster growth process which is in line with the balance of plant height and large plant stem.

Besides, because of the influence of exogenous triacantanol, there was also a possibility due to the influence of internal factors such as seed vigor, seed germination, and aging cacao pods. The internal factors would affect plant growth better. However, higher concentrations of triacantanol (2.0 ml/L) may be able to respond to a cell or tissue of cacao seeds to produce a certain compound that could inhibit plant growth.

According to Salisbury and Ross (1995) these compounds interfere with DNA transcription and RNA translation so great, so that the enzymes necessary to coordinate the growth is not generated properly. Hoagland (1980) also found that triacantanol could also selectively inhibit growth at higher concentrations.

CONCLUSION

Different concentrations of triacantanol significantly affected the growth of cacao seedlings at the age of 14 weeks. The best triacantanol concentration was a concentration of 1.0 ml/L, which was able to increase the growth of cacao seedlings: seedling height, number of leaves, length of leaves, and diameter of stem up to respectively 26.21%; 3.70%; 42.28%; and 10.42% higher than the controls at the age of 14 weeks.

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Rama R. Sitinjak and Dingse Pandiangan: *The Effect of Plant Growth Regulator Triacantanol*.....

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