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The Response of Growth on Shoot Cuttings and Stem Cuttings of *Citrus amblycarpa* L. after Giving Atonik

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ABSTRACT

Citrus amblycarpa L. much useful in human life, both as a cooking spices and as an ingredient in curing various diseases. This plant propagation by cuttings, in addition to producing excellence parent, also bear fruit quickly. Vegetative propagation by cuttings can be improved by using growth regulators. Atonik is one of growth regulators are able to increase the growth of cuttings of the plant. This study aims to determine the best growth response of shoot on the shoot cuttings and stem cuttings of the *Citrus amblycarpa* L. after giving of Atonik growth regulators. The design used was design completely randomized with factorial pattern. The first factor is the concentration Atonik with level: 0 ml L^{-1}, 10 ml L^{-1}, 30 ml L^{-1}, 60 ml L^{-1}, and 120 ml L^{-1}. The second factor is the type of cuttings consists of: shoot cuttings and stem cuttings. Data were analyzed by using test Duncan Multiple Range Test (DMRT) at 5% confidence level. The analysis showed that the giving 10 ml L^{-1} Atonik can enhance the best growth response up to be able to reach about 3.37 cm shoot height and 3.63 cm long leaf on the shoot cuttings. While on stem cuttings, required the optimum concentration (60 ml L^{-1}) Atonik to be able to obtain the best growth response, about 2.7 cm high buds, leaf length of 2.8 cm, and 2.8 number of buds on the 4th week after planting. To obtain the best growth response of shoots on the shoot cuttings required Atonik with lower concentrations when compared to the stem cuttings of *Citrus amblycarpa* L.

Keywords: Atonik, *Citrus amblycarpa* L., Shoot cuttings, Stem cuttings, Vegetative.

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INTRODUCTION

Citrus amblycarpa L. is a kind of the sour orange that is often called the orange chili sauce, which in Malay called Limau citrus. The sour orange is one kind of garden plants that used by housewives mainly as a spice in cooking, to flavor chili sauce because its fruit aroma. Its fruit water used to soak meat in order savory aroma, but its leaves are also used to flavor fried side dishes [1].

All kinds of citrus have essential oils containing D-limonene as a major component, including *Citrus amblycarpa* L. has antioxidant activity of 4% of all the essential oils and antimicrobial activity with inhibition of 10 mm against Gram-positive bacteria and 8 mm against Gram-negative bacteria [2]. The food intake of citrus limonoids may provide a protective effect against several types of cancer [3], for example, colon cancer [4]. Citrus also contain other bioactive components, such as vitamin C, β-carotene, flavonoids, folic acid, and dietary fiber. A high intake of citrus fruits can reduce the risk of degenerative diseases [5]. Citrus also contain phenolics, flavonoids, and hesperidin [6, 7], flavanone-O-glycoside and flavones-O- or -C-glycosides [8] that potentially as a source of natural antioxidants [6]. For the consumption of citrus fruit recommended by all the edible tissues [9]. Consumption of citrus can improve antioxidant status index, and the possibility of cardiovascular health and insulin sensitivity [10]. This Citrus is also an ideal component of a low-fat, and sodium restricted diet [11]. The need for this citrus crop production will increase with increasing public attention to the world of health. Therefore, efforts should be made to compensate for consumer demand, which is increasing in line with increasing population density.

Availability of this sour orange is still lacking, the available amount is the amount of citrus fruit crop production. So that community needs can be fulfilled, the plant is generally propagated by generative via seeds. But in this way, excellence the parent of this plant can not be derived 100% towards its derivatives, and long enough time to bear fruit about 10 years or more. To obtain offspring expected by the community 100% can be obtained by vegetative propagation, and will bear fruit in 2-3 years [12]. One type of propagation by vegetative means is through the multiplication of shoot cuttings and stem cuttings using growth regulators. Plant growth regulators are organic compounds that can stimulate, inhibit, or modify the physiology and morphology, as well as modify plant growth and development, which is biologically active at very low concentrations and elicit responses similar to those observed on the plant hormone [13].

Growth regulator in the right conditions to be able to increase growth and crop production. Growth regulator type of auxin has the potential to stimulate the growth of shoots and roots on vegetative propagation of various types of plants including some plant species *Citrus sp*., among others growth regulators acid indole-3-butyric (IBA) effectively enhance the growth of stem cuttings *Citrus auriantifolia* [14], stem cuttings medial of *Citrus medica* Linnaeus Corsian cultivar [15], semi-hard wood cuttings of *Citrus* [16], semi-hard wood cuttings of *Citrus sinensis* L. Osbeck [17], semi-hard wood cuttings of *Citrus* kind Sunki mandarin hybrid [18], shoot cuttings of *Citrus lemon* [19], and shoot cuttings of *Citrus limon* (L.) Burm. and *Citrus meyearii* Y. Tan. [20], as well as the effect on the initiation of the growth capacity of stem cuttings *Citrus* [21]. This suggests that the growth regulator auxin potential to stimulate the growth of shoots and roots on vegetative propagation of plants *Citrus sp*.

Atonik is a synthetic growth regulators of the auxin group that is able to stimulate the growth of shoot cuttings and stem cuttings of plants. This growth regulator is biostimulant based nitrofenolate, which produces a variety of formulations that positively affect the vital processes of plants and the effect is usually more pronounced under stress, and that the species of the plant economically important growing under controlled conditions [22, 23]. Giving Atonik to plants can stimulate the growth and development of the generative better, also the status of water and membrane integrity, reducing the negative effects of stress, gives a better tolerance for the performance of the plants that are not profitable, improve the quality and quantity of crops, fruits, and vegetables [23].

Several studies have successfully demonstrated a variety of potential possessed by growth regulator substances Atonik on various plants, such as: can increase the amount of fruit on tomato plants [24], improved yields in cotton crops [25], improve the quality of rooting in cuttings of sugar beet [26], promote growth and development (up inflorescences) in plants motherwort (*Leonurus cardiaca* L.) [27], on *Thymus vulgaris* L. [28], and the hydrangeas [29], can increase vegetative growth on *Vicia faba* L. [30], on the shoot cuttings of *Theobroma cacao* L. [31] and on the stem cuttings of *Rosa sp.* [32], affect the essential oil content and improve

potions results [33], can improve glucose, sucrose, polysaccharides, ammonia, amino, total nitrogen and protein in tomato plants [34]. However Atonik (1, 2, 3 g) had no effect in stimulating rhizogenesis on rooting of shoot cuttings from semi-timber species *Karwinskia humboldtiana* (Roem et Schut) Zucc. and *Karwinskia parvifolia* Rose. It depends on the age of the plant, the type of stimulator, cuttings, substrate, and growing conditions [35].

Due to vegetative propagation by cuttings technique mainly using shoot cuttings and stem cuttings of *Citrus amblycarpa* L. has never been done, and to offset the increasing market demand for the production of kinds of citrus, so this research should be conducted to determine the response of the growth of shoots on the shoot cuttings and stem cuttings of *Citrus amblycarpa* L. when propagated by giving Atonik.

MATERIALS AND METHODS

The research was conducted from November 2015 until February 2016, in greenhouse farming communities, villages Perdagangan, Simalungun District. The materials used are mainly shoot cuttings and stem cuttings of sour orange *Citrus amblycarpa* L., Atonik, planting media, water, etc. The tools are mainly used picks, shovels, polybag, scales, handsprayer, gauges, vernier caliper, documentation, etc. Working procedures: shoot cuttings and stem cuttings of citrus Limau provided. Planting medium consisting of manure: sand: soil = 1: 1: 1 is inserted into a polybag. Shoot cuttings and stem cuttings dipped in Atonik with a predetermined concentration. Polybags filled with shoot cuttings and stem cuttings accordance with the treatment, arranged randomly. In cuttings given Furadan 3G, and 0.1% Dithane M45.

This study uses a completely randomized factorial design with three replications. The first factor is the concentration Atonik with 5 levels ie 0 ml L^{-1} (A0), 10 ml L^{-1} (A1), 30 ml L^{-1} (A2), 60 ml L^{-1} (A3), and 120 ml L^{-1} (A4), and the second factor is the type of cuttings, namely shoots cuttings (P) and stem cuttings (B). The entire treatment amounted to 10, and each treatment there are five cuttings, then the total number of samples in this study was 150 samples. The growth parameters on shoot cuttings are shoot height, leaf length and number of leaves. The growth parameters on stem cuttings are shoot height, number of buds, number of leaves, and leaf length. To determine the effect of treatment towards the parameters tested, the observed data were analyzed by F test, while to know the difference between treatments, performed further tests by using test Duncan Multiple Range Test (DMRT) with a significant 5% [36].

RESULTS

The results demonstrate that Atonik to the growth of shoots on shoot cuttings and stem cuttings of *Citrus amblycarpa* L. affect the growth response of shoot height and leaf length, but does not affect the number of leaves on the 4th week after planting. The giving of Atonik towards stem cuttings greatly affect the number of buds on the 4th week after planting.

The results of the analysis of Duncan Multiple Range Test (DMRT) showed that giving 10 ml L^{-1} Atonik to the shoot cuttings of *Citrus amblycarpa* L. effectively enhance the best growth response of shoot height, which can reach about 3.37 cm (25.92 times higher than control) when compared with no giving Atonik and other treatment, but did not differ significantly on giving 30 ml L^{-1} and 60 ml L^{-1} Atonik on stem cuttings. On stem cuttings, giving 60 ml L^{-1} Atonik effectively enhance the best growth response of shoot height, which can reach about 2.7 cm. This shows that to improve the response of growth high buds on the stem cuttings are needed Atonik effective concentrations higher than 10 ml L^{-1} Atonik or required optimum concentration of 60 ml L^{-1} Atonik. This treatment did not differ significantly on giving 30 ml L^{-1} Atonik on stem cuttings, but significantly different with stem cuttings were not given Atonik or other treatment, as well as to the treatment given to shoot cuttings except the concentration of 10 ml L^{-1} Atonik. This can be seen more clearly in **Table 1**.

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Table 1. The influence of Atonik on the growth of shoots in the shoot cuttings and stem cuttings of *Citrus amblycarpa* L. aged 4 weeks after planting.

No	Treatments	The average of parameter		
		Buds high (cm)	Long leaf (cm)	The number of buds
1	Shoot cuttings (RA0)	0,13 c	0,2 c	
2	Shoot cuttings + 10 ml L^{-1} atonik (RA1)	3,37 a	3,63 a	
3	Shoot cuttings + 30 ml L^{-1} atonik (RA2)	0,8 c	0,97 bc	
4	Shoot cuttings + 60 ml L^{-1} atonik (RA3)	0,3 c	0,17 c	
5	Shoot cuttings + 120 ml L^{-1} atonik (RA4)	0,17 c	0,0 c	
6	Stem cuttings (SA0)	0,1 c	0,1 c	0,1 c
7	Stem cuttings + 10 ml L^{-1} atonik (SA1)	0,23 c	0,5 c	0,47 c
8	Stem cuttings + 30 ml L^{-1} atonik (SA2)	2,1 abc	1,07 bc	0,93 bc
9	Stem cuttings + 60 ml L^{-1} atonik (SA3)	2,7 ab	2,8 ab	2,8 a
10	Stem cuttings + 120 ml L^{-1} atonik (SA4)	0,43 c	0,33 c	0,27 c

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

Application 10 ml L^{-1} Atonik also effectively enhance the growth response of leaf length best on shoot cuttings of *Citrus amblycarpa* to be able to obtain the highest leaf length of about 3.63 cm per shoot (18.15 times higher than the control) at 4th weeks after planting when compared to other treatments given to shoot cuttings and stem cuttings given to the exception of 60 ml L^{-1} Atonik. The difference of growth response shoot cuttings which given 10 ml/L Atonik and which not given Atonik (control) aged 4 weeks can be seen more clearly in **Figure 1**.

Application of 60 ml L^{-1} Atonik effectively enhance the growth response of leaf length on stem cuttings of *Citrus amblycarpa,* up to be able to obtain the highest leaf length of about 2.8 cm per buds (28 times higher than the control) at week 4 after planting. This treatment was significantly different compared to control and other treatments on stem cuttings. Best growth response resulting from the application of 60 ml L^{-1} Atonik on stem cuttings of *Citrus amblycarpa* that can effectively stimulate shoot height, leaf length, and number of buds at the age of 4 weeks after planting, can be seen in **Figure 2**. Application of 60 ml/L Atonik on stem cuttings, also can effectively enhance the best growth response up to be able to obtain the highest number of buds about 2.8 buds per stem cuttings. This treatment is more effective than control and other treatments on stem cuttings. Application of Atonik with a high concentration (120 ml L^{-1}) can cause pressure on the growth response on shoot cuttings and stem cuttings of this Citrus. Growth response on shoot cuttings and stem cuttings of *Citrus amblycarpa* L. after giving of Atonik at week 4 after planting, can be more clearly seen in **Figure 3.**

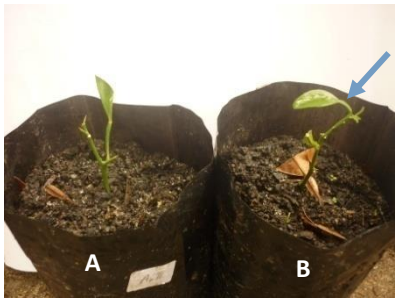

Fig.1. Shoot cuttings of *Citrus amblycarpa* L. age of 4 weeks: A, Treatment control 0.0 ml L^{-1}. B, Treatment of 10 ml L^{-1} Atonik.

Fig. 2. Stem cuttings of *Citrus amblycarpa* L. age of 4 weeks : the best treatment was 60 ml L^{-1} Atonik.

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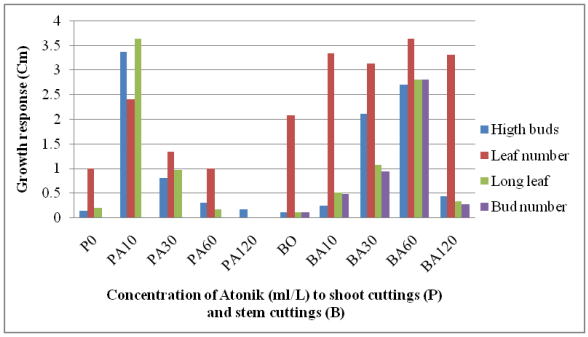

Fig. 3. The growth response of shoot cuttings (P) and stem cuttings (B) of *Citrus amblycarpa* L. to Atonik at 4th weeks.

DISCUSSION

Atonik (Asahi Co., Ltd.; Nara Prefecture, Japan) is a commercially available product containing the active ingredient, Natrium 5-nitroguaiacolate ($NaC_7H_6NO_4$) 1.25 g/L, Natrium ortho-nitrophenolate ($NaC_6H_4NO_3$) 2.5 g/L, and Natrium para-nitrophenolate ($NaC_6H_4NO_3$) 3.75 g/L [37]. The active ingredient, called nitrophenolates, which is found naturally in plants and stimulate plant growth by changing the activity of certain antioxidant enzymes [24], such as superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaicol peroxidase, GOPX and glutathione-S-transferase, GST). This enzyme is very efficient as an antioxidant defense systems that work in controlling cascades against uncontrolled oxidation and protect the plant's cells from oxidative damage by catching Reactive Oxygen Species (ROS). Reactive Oxygen Species (ROS) consist of free radicals ($O_2^{\cdot-}$, superoxide radicals, $OH^{\cdot}$, hydroxyl radical; $HO_2^{\cdot}$, perhydroxy radical and $RO^{\cdot}$, alkoxy radicals) and non-radical form (mulekular) (H_2O_2, hydrogen peroxide dan $^1O_2$, singlet oxygen) [38]. Some of Reactive Oxygen Species (ROS) continues to be produced in the plant as a product of many metabolic reactions, such as photosynthesis, photorespiration and respiration. Reactive Oxygen Species can oxidize all types of cellular components, including proteins, fats, carbohydrates, and DNA which ultimately lead to oxidative stress [39, 40, 41, 38]. Some of ROS are highly toxic and rapidly detoxified by various cellular mechanisms of enzymatic and non-enzymatic. Oxidative stress occurs when there is an imbalance between ROS production and antioxidant defenses [39], or due to the depletion of antioxidants and ROS increases [42]. According to Gill and Tuteja [38] over production of ROS in plants can also be caused by various abiotic stresses, such as temperature stress [43], and cadmium (Cd) [44].

Similarly in this study, shoot cuttings and stem cuttings of plants *Citrus amblycarpa* were grown in growth media with the application of Atonik growth regulators. The tissue of cuttings of this plant, in addition to attempted repair itself by forming new cells replace the cells damaged by cuts, will also seek to protect themselves against microbial infections and various abiotic stresses. At the time of this condition, the metabolic system in the body of plant cuttings strive to be able offset of the conditions around tissue which is still injured, the defense system becomes more active in attempts to balance ROS generated from the metabolic system with the antioxidant defense system that seeks to keep pressing the production of ROS, ROS affect the expression of a number of genes, because of controlling several processes such as growth, cell cycle, programmed cell death (PCD) or necrosis, in response to abiotic stresses, pathogen defense, systemic signaling and development [38, 45]. According to Ahmad *et al*. [39] ROS participate in signal transduction, but also modify cellular components and cause damage. To repair this condition and to enhance the growth response of shoot cuttings and stem cuttings of *Citrus amblycarpa*, required the addition of an active ingredient namely Atonik. Atonik as biostimulator which can alter the activity of certain antioxidant enzymes in stimulating the formation of cells and new tissue to replace the damaged tissue, and simultaneously to accelerate the growth and development of the shoot cuttings and stem cuttings of Limau citrus with the excessive ROS arrest. Response of growth that starts from the process of cell differentiation in the formation of cells and new tissue, then proceed to the process of morphogenesis in forming part of plant organs including buds, stems, and leaves, of course it takes concentration Atonik effective so that it can achieve the growth and development of the shoot cuttings and stem cuttings of *Citrus amblycarpa* optimally.

In this study, application of 10 ml/L Atonik on shoot cuttings of *Citrus amblycarpa* can effectively enhance the best growth response compared with all treatments PGR Atonik, and with the treatments on stem cuttings except at a concentration of 60 ml L^{-1} and 30 ml L^{-1} Atonik. When viewed from the growth response of shoot cuttings were given Atonik, it can be said that the applicaion above 10 ml L^{-1} Atonik on shoot cuttings will inhibit the growth response of shoots (**Figure 3**). Application 10 ml L^{-1} Atonik on the shoot cuttings of this citrus, can effectively increase the height shoots up to an average of 3.37 cm (25.92 times higher than the control) at 4th weeks after planting. This Atonik concentration is effective in increasing the likelihood of antioxidant enzyme activity and auxin levels to increase the number and cell elongation, so as to increase of the growth response speed of shoot on the shoot cuttings of *Citrus amblycarpa* L. Because of the possibility of the hormone auxin in the shoot higher than the trunk area, so it to stimulate the growth of shoots on the shoot cuttings only needs a bit of growth regulators. Endogenous and exogenous hormones work together to stimulate vegetative growth and development in plants, also on the shoot cuttings and stem cuttings of plants *Citrus amblycarpa* L. Conversely the higher the concentration of hormones will increasingly suppress the occurrence of plant growth and development. Therefore, the application of concentration of 30-120 ml/L Atonik, would indicate height of shoots were low compared to the concentration of 10 ml L^{-1} Atonik on shoot cuttings of *Citrus amblycarpa*. Application 10 ml L^{-1} Atonik on the shoot cuttings of *Citrus amblycarpa* thought to potentially increase cell growth in bud formation by altering the activity of antioxidant enzymes as an efficient defense system. According to Cabisco *et al*. [41] that living organisms must establish mechanisms to protect themselves against oxidative stress with enzymes such as catalase and superoxide dismutase. Enzyme of superoxide dismutases (sods) can convert superoxide to hydrogen peroxide, were is then removed by glutathione peroxidase or catalase. Thereby, sods prevent the formation of ROS were highly aggressive [46]. Djanaguiraman *et al.* [24] also showed that the application of nitrophenol (Atonik) significantly increase the activity of antioxidant enzymes, such as superoxide dimutase (SOD), catalase (CAT), peroxidase (POX), and the content of auxin is accompanied by decreased activity of polyphenols aksidase (PPO) enzyme IAA oxidase (IAAO). Then according to Gara *et al.* [47] redox enzyme activity during the hypersensitive response program, does not seem to be a consequence of oxidative stress caused by the production of ROS were great, but induced as part of the transduction pathway triggering defense responses and programmed cell death (PCD). Fleury *et al.* [48] showed that ROS molecules are not only harmful to the cells, but also have a physiological role as a mediator in signal transduction pathways. The important role of ROS in physiological may be seen on: (a) an increase in ROS levels during cell differentiation, (b) the existence of the ROS-producing enzymes such as NADPH oxidase (NOX), (c) the involvement of NOx in the developmental process, and (d) the conservation of the signal-transduction mechanisms. This indicates manipulation of reactive species, as a strategy to regulate cell differentiation [49]. Similarly, at the initiation stage of growth response of shoot cuttings and stem cuttings *Citrus amblycarpa*, treatment of 10 ml L^{-1} Atonik can effectively increase the activity of enzymes in the cell differentiation process towards the establishment and growth of shoots to form the stems and leaves. According to Laloi *et al.* [50] a variety of plant responses induced by hydrogen peroxide produced by a genetically controlled manner by NADPH oxidase. Changes the concentration of ROS into signals that alter gene transcription. This means that to improving the growth response in shoot cuttings and stem cuttings of *Citrus amblycarpa*, especially when regulation of cell differentiation, there is very close cooperation between the role Atonik as biostimulator, and the important role of ROS as a mediator in signal transduction in the plant physiological processes.

This study also shows that in addition to PGR concentration, type of cuttings also affect the growth response of cuttings in vegetative propagation of *Citrus amblycarpa* L. To obtain the highest average shoot height (2.7 cm) on the stem cuttings are needed Atonik higher concentration (60 ml L^{-1}) compared to the shoot cuttings (10 ml L^{-1}). Because of the possibility of oxidative stress higher occur in the stem cuttings due to cell metabolism is more active, so that required concentration of active ingredient Atonik higher (60 ml L^{-1}) to be able stimulate the differentiation of cells by increasing the activity of antioxidant enzymes and increase defense system for suppressing the production of ROS and interact with endogenous auxin in cooperation with other hormones, especially cytokinin to induce bud formation in some places and growth through a process of morphogenesis in stem cuttings *Citrus amblycarpa* L. According to Tezuka *et al*. [51] Auxin were synthesized de novo from lateral branches inhibits shoot regeneration after cutting and endogenous cytokines be able stimulate the regeneration of shoots. Djanaguiraman *et al*. [52] also showed that plants were sprayed with Atonik or combination with pesticides, have no symptoms fitotoxic. Atonik 0.1% be able reduce the potential diseases in plants up to 3-7 times lower than the control plants [53]. Atonik is biostimulator which can reduce severity of late blight and early cancer in potato tubers [54]. So Atonik application with the optimum concentration (60 ml L^{-1}) on the stem cuttings of *Citrus amblycarpa*, in addition to potentially improving the

growth response of shoot, also has the potential to improve defense system that can protect the tissue cuttings of various biotic and abiotic pressures high enough. According to Mate's [40] a variety of antioxidants exert their effects by catching superoxide, or by activating detoxification. Under normal conditions, the antioxidant system of the cell to minimize disruption caused by ROS. When ROS increased to a level that overcomes cellular antioxidant, the result is oxidative stress. Intracellular ROS play a role as a mediator of normal and pathological signal transduction pathways [55]. Reactive oxygen species play an important role in the early stages and the final stage of the plant pathogenesis response, as cellular signaling molecules and direct antipatogen agent [56]. Antioxidant enzymes, antioxidants and enzymes that produce ROS, responsible for maintaining the level of ROS in under strict control. This allows ROS to serve as a signaling molecule to coordinate the various processes in the plant. Specificity of the biological response to the ROS depending on the chemical identity of ROS, the signal intensity, the production site, stage of plant development, the pressure faced before, and interaction with signaling other molecules such as nitric oxide, lipid messenger and plant hormones [57]. The steady state level of ROS in the cells needs to be strictly regulated. As in Arabidopsis, the network of at least 152 genes involved in managing the level of ROS. These networks are highly dynamic and encode arrest ROS, and a protein that produces ROS [58]. According to Moradas *et al.* [59] a number of primary antioxidant activity and metal ion homeostasis system or detoxification contribute to the protection of oxidative stress. The response of oxidative stress involves the response of transcription factor for peroxide, superoxide anions and metal ions. Likewise in suppressing the production of ROS in the tissues of stem cuttings of *Citrus amblycarpa*, a number of antioxidant activity and system homeostasis or detoxification as well as a number of activities of antioxidant enzymes contribute in defense system for protection of oxidative stress so the cells that formed has the potential to enhance the growth of shoots to form stem and leaves, as a sign that the plant cuttings have been able to adapt. Temple *et al*. [60] also said that the response patterns of transcription in particular depending on the concentration of ROS and antioxidant systems 'classic' caused by high concentrations of ROS can be suppressed when cells adapt to low concentrations of ROS.

In this study, that in order to improving response of the best growth at shoot cuttings required 10 ml L^{-1} Atonik and on stem cuttings required 60 ml L^{-1} Atonik. This shows that Atonik with a concentration of this is a compounds more effective in activating the enzyme in the cell so as to accelerate the plasma flow of cells that cause all the cells of plants on the physiology of plant shoot is going well, parts of the plant vegetative will grow faster and stronger. Atonik growth regulator is very beneficial to stimulate all the organs of the plant by the use of different concentrations as needed. Atonik consisting essentially of synthetic auxin that will promote division, enlargement and extension cells by way of ion pump activation on the plasma membrane. The cell walls become loose and cause pressure on the cell walls to be reduced. Water easily enter the cell, resulting in the enlargement and extension of cells. Plant hormones regulate many aspects of plant growth and development. Auxin, one fitohormon to control some of the significant developments, such as the formation and control of the shoot apical meristem and root apical meristem as well as play a role in organ regeneration [61]. According to Perrot-Rechenmann [62] fitohormon are the primary regulator of cellular processes and development in relation to a certain threshold concentration and the response of cells or tissues specifically. Auxin concentration gradient is the driving force for organogenesis and pattern, pointing Auxin as morphogens plants. Pool of plant stem cells, the source for all the organs, which were first determined. Auxin along with cytokines interact to control the regeneration of organs, as occurs in stem cuttings or cuttings shoots. Auxin is essential for root-stem cell specification. Antagonistic interaction between auxin and cytokinin is important to determine the root-stem cell niche first [63]. As with any application Atonik and BA could affect vegetative growth criteria and elements of biochemistry [64]. In plants, every process of development will integrate signaling networks regulated by fitohormon different, and the interaction between hormonal pathways important for regulating the effect [65]. Continuous growth of the stem is controlled by the coordinated work by some fitohormon, including auxin. The effects of hormones on growth stems largely mediated by the regulation of auxin biosynthesis and transport dependent local auxin distribution. According to Yang *et al*. [66] stem elongation can be stimulated by the hormone auxin indoleacetic acid (IAA) in collaboration with Gibberellins (GA). IAA cause a very rapid growth rate, GA enhance the growth response gradually over time through an increase in the length and number of cells. GA can increase auxin induction of cell elongation but can not stimulate the elongation of the absence of auxin. The effect of GA may, in part, mediated by auxin. Auxin and GA separately control the processes that together contribute to stem elongation. Inhibition of or increase in growth can be caused by a deficiency or excess of the concentration of endogenous hormones, particularly endogenous auxin.

In this study also, the application of 10 ml L^{-1} Atonik effectively enhance the growth response of leaf length in the shoot cuttings of C*itrus amblycarpa*, which may cause the shoot cuttings can achieve the highest leaf length of about 3.63 cm per shoots at 4th weeks after planting when compared with control and other treatments, and the treatment given to the stem cuttings except giving 60 ml L^{-1} Atonik. Then, the application Atonik with a high concentration (120 ml L^{-1}) can cause pressure on the growth response of leaf length, on a shoot cuttings and stem cuttings of C*itrus amblycarpa*. This suggests that in improving growth response of leaf length at *Citrus amblycarpa* cuttings affected besides the concentration of PGR, also the type of plant cuttings. This suggests that the mechanism of action of Atonik as biostimulator besides important role in improving the defense system also increases hormones work in the *Citrus amblycarpa* cuttings specific and coordinated so that the effect of certain growth including the response of the extension leaf. According to Przybysz *et al*. [67] Atonik stimulate vital processes such as plant growth and development, affecting the physiology and biochemistry, which often lead to an increase in biomass accumulation. Plants supplied with this Atonik will be higher and more developed. Larger biomass accumulation after application biostimulator mainly due to the efficiency of the photosynthetic apparatus is better, which is realized by (i) greater leaf area, (ii) total chlorophyll content were higher and (iii) an increase in the intensity of photosynthesis. Application Atonik in plants can also be effective and useful when grown under optimal conditions. Such as *A. thaliana* plants are supplied with Atonik has a wider leaf area, chlorophyll content, the intensity of photosynthesis and chlorophyll fluorescence values are higher [22]. According to Frerichs [23] plant supplied with Atonik show growth and better development, biomass accumulation, and higher efficiency in the photosynthetic apparatus, the status of water and membrane integrity. Atonik reduce the negative effects of stress, gives a better tolerance for plant growth conditions are not favorable. Similarly the application of 10 ml L^{-1} Atonik effectively enhance the growth response, including the shoot height, and length of leaves on the shoot cuttings, while the application of 60 ml L^{-1} Atonik effectively enhance the growth response, including the shoot height, leaf length, and number of buds on the stem cuttings of *Citrus amblycarpa* L. This study shows that to obtain the best vegetative growth of the shoot cuttings and stem cuttings of plants *Citrus sp*. mainly on the type of *Citrus amblycarpa* is required Atonik with different concentrations. Atonik concentration that effective to increase vegetative growth at shoot cuttings are lower when compared to the stem cuttings.

CONCLUSION

Applications Atonik to the growth of shoots from shoot cuttings and stem cuttings of *Citrus amblycarpa* L. is the positive effect on the growth response of buds height and leaf length, but not to number of leaves. Applicaton Atonik to stem cuttings greatly affect the number of buds on the 4th weeks after planting. The treatment of 10 ml L^{-1} Atonik to shoot cuttings effectively improve the response of high buds (3.37 cm) and leaf length (3.63 cm) when compared with those without atonik (control) or with other treatments. While on stem cuttings, application 60 ml L^{-1} Atonik effective to improve the response of high buds (2.7 cm), leaf length (2.8 cm), and number of buds (2,8 buds per stem cuttings) when compared with controls and other treatments.In improving vegetative growth of the shoot cuttings and stem cuttings of plants *Citrus sp*, especially on the type of *Citrus amblycarpa* L. is required Atonik with different concentrations. Atonik concentration that effective to increase vegetative growth at shoot cuttings lower compared to the stem cuttings.

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