Improvement growth of sapodilla (Achras zapota L.) by chitosan

E Yuniastuti1*, Q Waliyyudin2, Nandariyah3 and M N I Delfianti4

1 Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
2 Undergraduated School, Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
3 Center of Plant Breeder, Research and Development, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
4 Graduate School, Department of Agronomy, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia

Corresponding author: yuniastutisibuea@staff.uns.ac.id

Abstract. Indonesia’s palm oil production needs to be developed; generative sapodilla cultivation has a very long growth. The effort that can be done to increase the growth of sapodilla seeds for the provision of quality seedlings. Giving chitosan expected to increase the growth of sapodilla seeds because there is chitosan content that accelerate plant growth, and age planting shorter. The aim of this research is to know the concentration and time of spraying of chitosan to be applied to generative sapodilla seeds. The study used Randomized Complete Block Design (RAKL) of 2 factors. The first factor was chitosan concentration consisting of four levels (0, 2, 4, and 6 mL/L) and the spraying time consisted of three levels (morning, afternoon, and morning and afternoon), each treatment combination was repeated three times. that the concentration of chitosan has significant effect to the leaf area with the best treatment at 6 mL/L concentration and the time of spraying treatment had significant effect on the increase of the number of branches with the best treatment during the morning spraying.

1. Introduction
The production of sapodilla in Indonesia from 2012 to 2016 are 135.332; 127.690; 138.209; 134.647 and 132.284 tons [1,2]. Sapodilla has many benefits, in addition to greening and furniture materials, sapodilla has a high nutritional value, many contain powerful antioxidants for people with diabetes mellitus [3–5]. The increase and decrease as well as the many benefits of sapodilla is the reason that it has the potential to be developed in Indonesia.

Sapodilla fruit breeds generative and vegetative communities. Generative cultivation has advantages such as stronger roots that can be used as rootstock of cultivation with graft. Weakness of growth of seeds of sapodilla seeds is considered slow and longer to bear fruit. The growth of seedlings of this sapodilla can be supported by the use of growth regulator substances. ZPT is a hormone that can affect plant physiology. One type of ZPT is chitosan. Chitosan is one type of ZPT from the utilization of shrimp leather waste processed with gamma radiation and electron beam to oligochitosan [6–8]. According to
BATAN [6], Yuniastuti et al [9] and Zeng et al [10] chitosan can accelerate plant growth, increase yield and shorter planting time.

The success factor of ZPT usage is the concentration and the supporting environmental conditions. The proper use of ZPT concentrations can affect the flowering process of the plant. Environmental conditions in accordance with the needs will support the plant in the process of absorption of ZPT, thus need to be conducted research to know the concentration and time of spraying good chitosan on growth of sapodilla seeds in order to accelerate the growth of sapodilla seedlings in Indonesia.

2. Methods
This research was conducted from April to September 2017 at Greenhouse Faculty of Agriculture, Sebelas Maret University. The tools used are polybag, paper label, hand sprayer, clear plastic, measuring cup, ruler, camera, stationery, preparation, microscope. The material used is sapodilla seedlings (Achras zapota) from seeds aged 2 years, chitosan, planting media in the form of soil, compost and sand (1:1:1), clear nodes for stomata condition observation. This research consists of two factors arranged in the Design Randomized Complete Group (DRCG). The first factor is the concentration of chitosan consisting of four levels i.e. 0; 2; 4; and 6 mL/L. The second factor is the spraying time consisting of three levels i.e. the morning; afternoon, and morning and evening. Each treatment combination was repeated three times. The results were analyzed using Analysis of Variance (ANOVA) with 5% level and if the real difference was continued with Duncan's Multiple Range Test (DMRT). Data analysis was done using SPSS software.

3. Results and discussion

3.1. Plant height
Plant height is often used as an indicator of growth as well as parameters in measuring the effect of treated treatment. Based on the analysis of variance at 12 MST showed that treatment with the concentration of chitosan have no significant effect on plant height with significance value 0.071. Treatment time of spraying have no significant effect on plant height with significance value 0.994. The interaction between concentration and spraying time of chitosan have no significant effect to plant height.

| Concentration | Times to spray | Average |
|---------------|----------------|---------|
|               | Morning (W1)   | Afternoon (W2) | Morning and afternoon (W3) |       |
| 0 mL/L (K1)   | 7.87           | 4.37     | 5.90       | 6.04a |
| 2 mL/L (K2)   | 6.60           | 4.93     | 6.40       | 5.98a |
| 4 mL/L (K3)   | 8.03           | 5.10     | 8.13       | 7.09a |
| 6 mL/L (K4)   | 5.77           | 10.87    | 6.53       | 7.72a |
| Average       | 7.07a          | 6.32a    | 6.74a      |       |

The numbers followed by different letters in the same columns and rows are significantly different in the 5% DMRT

The highest average plant height is the treatment of concentration with 6 mL/L because the hormone content contained in it more when compared with others, so the plants give a good response. The treatment of spraying time in the morning has the greatest average compared to afternoon and morning + afternoon spraying. Application of chitosan with the right time and manner will encourage apical meristems to grow. According to research Suptidjah et al [11], spraying chitosan directly on the shoots and leaves of plants can increase the synthesis of auxin in tomatoes. The process of auxin synthesis is present in the apical meristem, so that the activity of the hormone that exists will encourage the growth of the sapodilla seedlings high.
3.2. Leaf amount

Leaves are organs that are able to bind the energy of sunlight through the process of photosynthesis and used as an indicator of plant growth. The following is an average increase in the number of leaves for 12 MST.

Table 2. Average addition of sawo leaf amount at 12 MST (strands)

| Concentration | Morning (W1) | Afternoon (W2) | Morning and afternoon (W3) | Average |
|---------------|--------------|----------------|---------------------------|---------|
| 0 mL/L (K1)   | 28.00        | 23.33          | 27.33                     | 26.22   |
| 2 mL/L (K2)   | 24.67        | 25.33          | 26.33                     | 25.44   |
| 4 mL/L (K3)   | 20.00        | 28.00          | 32.00                     | 26.67   |
| 6 mL/L (K4)   | 25.67        | 32.33          | 27.33                     | 28.44   |
| Average       | 24.58        | 27.25          | 28.25                     |         |

The numbers followed by different letters in the same columns and rows are significantly different in the 5% DMRT.

Based on the result of variance analysis showed that the treatment with the concentration of chitosan have no significant effect on the number of leaves with the significance value of 0.537. Treatment time of spraying also no significant effect on the number of leaves with a significance value of 0.836. The interaction between the concentration treatment and the spraying time of chitosan also has no effect on the number of leaves. This is because the elements given for sapodilla only chitosan only. The content contained in the chitosan may if supported with other elements will be able to increase the addition of the number of leaves is also faster. Below is an incremental graph of each concentration treatment and spraying time of chitosan to the number of leaves.

Figure 2. shows the largest number of leaves increase at 6 mL/L concentration by spraying during the afternoon (32.33). According to Wanichpongpan et al [12], we can increase the growth factor on the length of the leaf stem, the rate of leaf growth, including the length and width of each leaf number of each shrub. The lowest number of leaves is at the treatment of 4 mL/L concentration with the time of morning spraying, this is because in addition to the input given to the plant, it could be the growth of the number of leaves that is accompanied by the leaves that fall from the plant. Figure 3 below shows the effect of adding chitosan to the number of leaves.
Figure 2. Influence of concentration and time of spraying of chitosan on sapodilla leaves (*Achras zapota*).

Figure 3. Differences in the number of leaves of control treatment (a) and addition of leaf amount of treatment chitosan 6 mL/L (b).

### 3.3. Number of branches

The number of branches is closely related to the number of leaves that are formed. The number of leaves will increase as the number of branches increases. Based on the analysis of the variety of treatment of concentration has no significant effect on the number of secondary branches with a significance value of 0.486 and the treatment of spraying time significantly affect the number of secondary branches with a significance value of 0.016. The interaction between the two treatments also had a significant effect on the number of secondary branches with a significance value of 0.021. The effect of concentration difference and time of spraying on the number of leaves can be seen in Figure 3.

#### Table 3. Number of branches sapodilla (*Achras zapota*)

| Concentration | Morning (W1) | Afternoon (W2) | Morning and afternoon (W3) | Average |
|---------------|--------------|----------------|-----------------------------|---------|
| 0 mL/L (K1)   | 5.33         | 3.33           | 1.00                        | 3.22<sup>a</sup> |
| 2 mL/L (K2)   | 4.33         | 4.00           | 2.67                        | 3.67<sup>a</sup> |
| 4 mL/L (K3)   | 4.33         | 1.00           | 2.67                        | 2.67<sup>a</sup> |
| 6 mL/L (K4)   | 2.00         | 4.33           | 2.33                        | 2.89<sup>a</sup> |
| **Average**   | **4.00<sup>b</sup>** | **3.17<sup>ab</sup>** | **2.17<sup>a</sup>** | **-** |

The numbers followed by different letters in the same columns and rows are significantly different in the 5% DMRT.
Based on table 3, the highest number of secondary branches is K1W1 (concentration 0 mL/L with morning spraying time) of 5.33 branches, while the lowest addition of secondary branch is K1W3 (0 mL/L concentration with morning and afternoon spraying time) and K3W2 (4 mL/L concentration with afternoon spraying time) of 1.00 branches. The treatment of chitosanic concentration has the largest mean of 2 mL/L concentration (3.67 branch) while the lowest mean is the concentration of 6 mL/L (2.67 branch). The highest average spraying treatment was in the morning (4.00 branches) and the lowest average of morning and afternoon spraying (2.17 branches). This is because in the morning the light received by plants to photosynthesize well enough so that the results of the photosynthesis process can be utilized for branch growth in sapodilla seeds. During the day the intensity of light is higher than the morning that resulted in environmental conditions also changed. The process of branch formation is influenced by the hormone development of the side buds are cytokines contained in chitosan. Chitosan-containing chitosan can increase growth, accelerate flowering, and increase productivity in some crops in agriculture, such as rice (Oryza sativa L.) [13], cucumber (Cucumis sativus L.) [14], and tomatoes (Lycopersicum esculentum Mill.) [15].

3.4. Leaf area (cm²)
Leaves are parts of plants that receive direct sunlight which is then exploited by plants for the process of photosynthesis. The area of the leaf will affect the rate of photosynthesis of plants. Also supported by Yuniastuti et al [3], Mulyani [16] and Yuniastuti [17] that the results of photosynthesis then distributed to all the organs of growth and development of plants. The measurement of leaf area in this research is by gravimetric method. Below is how to calculate leaf area. Below is sapodilla leaf area calculated at 12 MST.

| Concentration | Morning (W1) | Afternoon (W2) | Morning and afternoon (W3) | Average |
|---------------|--------------|----------------|-----------------------------|---------|
| 0 mL/L (K1)   | 23.74        | 26.18          | 29.07                       | 26.33a  |
| 2 mL/L (K2)   | 27.40        | 26.03          | 32.72                       | 28.72a  |
| 4 mL/L (K3)   | 34.09        | 29.83          | 29.83                       | 31.25a  |
| 6 mL/L (K4)   | 36.83        | 35.62          | 39.73                       | 37.39b  |
| Average       | 30.52a       | 29.41a         | 32.84a                      |         |

The number followed by different letters in the same column differs markedly on the DMRT 5%

Based on the analysis of variance, phytochemical concentration treatment had significant effect on leaf area with significance value 0.004, while the time treatment had no significant effect with
significance value 0.362. The interaction between the concentration of chitosan and the time of spraying treatment did not significantly affect the leaf area. The concentration treatment showed that the highest average leaf area was 6 mL/L (37.39 cm²), while the lowest was 0 mL/L (control). This proves that the higher concentration given to the leaves will increase the leaf area. Time treatment showed that the highest leaf area average was during the morning + afternoon (32.84 cm²) spraying and the lowest average leaf area on the afternoon spray (24.91 cm²). In the afternoon the stomata conditions of the leaves begin to narrow in order to reduce the presence of transpiration.

3.5. Leaves stomata
The condition of morning and evening stomata in this study also influenced the study. Stomata is part of the plant that regulates photosynthesis, transpiration and respiration. Stomata is commonly found in the epidermis of leaves almost 1 to 12% of the leaf surface. Stomata observation was performed by taking samples using a clear nan, taken with a clear soluble then placed on a preparation then observed under a microscope. Below is the stomata condition of the study.

| Concentration | Times to spray      | Average |
|---------------|---------------------|---------|
|               | Morning (W1)        | Afternoon(W2) | Morning and afternoon (W3) |         |
| 0 mL/L (K1)   | 146.67              | 144.33  | 148.67  | 146.56* |
| 2 mL/L (K2)   | 142.67              | 150.33  | 148.67  | 147.22* |
| 4 mL/L (K3)   | 150.33              | 152.50  | 160.33  | 154.39* |
| 6 mL/L (K4)   | 159.67              | 161.33  | 163.33  | 161.44* |
| Average       | 149.83*             | 152.13* | 155.25* |         |

The numbers followed by different letters on the same row and column are significantly different in the 5% DMRT.

Based on the analysis of variance, concentration and time treatment did not significantly affect the amount of leaf stomata. The interaction between the concentration of chitosan and the time of spraying treatment also had no significant effect on the amount of leaf stomata. The concentration treatment showed that the highest mean of the highest leaf stomata was 6 mL/L (161.44 stomata), while the lowest was 0 mL/L (control). Treatment of spraying time in the morning and afternoon has the highest average stomatal number of 155.25 stomata. In the afternoon the stomata conditions of the leaves begin to narrow in order to reduce the presence of transpiration. The number of stomata that opened during the morning + afternoon proved more abundant than others, this is because in the morning the intensity of light increases and the absorption of K+ ions and water with low CO2 concentration in the leaves that cause stomata to open. In the afternoon the plants tend to reduce the evaporation process so even though the photosynthesis process occurs but it is evident in the afternoon stomata leaves are still open. According to Khadijah [18], the morning temperature is still balanced with the body temperature of plants so that the evaporation of water is still controlled. Effect of chitosan so as to increase the nutrient absorption through leaf stomata, meaning the increasing number of chitosan supports stomata condition to keep doing the process of photosynthesis.

3.6. Leaf color
Leaf color is part of a substance reflected by light as a result of absorption of light on a particular wave. The green color of the leaves comes from chlorophyll or green substances contained in the leaves. According to Sumaenda [19] in chloroplasts there are major pigments of chlorophyll, carotenoids and xanthophyl contained in the thylakoid membrane. The chlorophyll present in this chloroplast then absorbs violet, blue and yellow light and reflects green. Checking the color of leaves of sapodilla plant is done by using a tool of Plant Tissue Color Book which then shows the notation of sapodilla leaves observed.

The leaf color of the research results showed 5GY 4/4 notation in Plant Tissue Color Book. This means with the dark green yellow with the notation 5 and the level of fading 4 and the level of loss also 4. Notation to record the color of leaves by showing the value of hue, value and chroma. This hue
notation shows its relation to the five color concepts which are then written in capital letters (e.g. G for green, etc.). Value denotes the degree of darkness of the color in relation to the scale extending from pure black symbols theoretically written 0 / to the pure white written 10 /, so that visually the color shown between pure black and pure white is written 5 / darker then the value of the number decreases below five. Chroma indicates the level of fading, sapodilla has a value 4. According Yuniastuti [20] the color of the lower surface of the palm leaf is pale green and the upper leaf surface is greener due to the difference in chlorophyll.

4. Conclusion
The conclusion obtained from the results of this study are treatment of chitosan concentration has an effect on sapodilla seed growth is on leaf area concentration 6 mL/L and treatment time to spraying chitosan have influence growth of seeds of sapodilla increasing number of branches on morning.

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