Shallot extract enhance root growth in crystal guava (Psidium guajava) stem cuttings

Sarjiyah*, D A Setiawan, I A Rineksane
Department of Agrotechnology, Faculty of Agriculture, University of Muhammadiyah Yogyakarta, Indonesia
*Email: sarjiyah@umy.ac.id

Abstract. The most efficient and effective propagation of guava plants is by using stem cuttings. The main obstacle in the multiplication of guava with stem cuttings is the root system. Plant Growth Regulators are needed to accelerate and increase the roots of guava stem cuttings to obtain quality seeds. Plant growth regulators that can be used to accelerate guava growth include Indole Butyric Acid, Naphtalene Acetic Acid. However, both growth regulators are not available at the farm level. One alternative material that can increase the growth of guava cuttings is shallot extract. Information is needed to find out what is the right concentration of shallot extract to get guava seedlings with high growth percentage and quality. The purpose of this study was to determine the exact concentration of shallot extract on the growth of guava root cuttings. The research method used was a single factor experimental which was arranged in a Completely Randomized Design. The treatments tested were without plant growth regulators (as a control), fresh shallot extract concentration of 1%, maceration of shallot extract concentrations of 1%, 2%, and 3%, IBA with a concentration of 500 ppm (as a comparison). Guava stem cuttings before planting are soaked in the treatment solution for 120 minutes. During seedling growth, observations of roots, stems and leaves are observed. Observation data were analyzed by analysis of variance and Duncan Multiple Range Test. The results showed that the concentration of maceration of shallot extract 2% was able to grow roots and shoots with the same quality as IBA concentration of 500 ppm on crystal stem guava stem cuttings.

1. Introduction
Crystal guava is a green to yellowish guava fruit with a round shape and sweet taste. Guava contains vitamin C 2-9 times higher than oranges and is believed to be able to cure dengue fever [1]. In addition, guava is also useful for protecting blood vessels and heart health and is useful for preventing cancer formation, maintaining healthy teeth, gums, capillaries, facilitating iron absorption and wound healing [2]. The production of guava in Indonesia from 2015 to 2017 fluctuated in numbers, namely: 62,815; 64,637 and 56,365 tonnes with the number of plants producing 2,151,534; 2,255,352 and 2,505,801 trees [3]. This indicates lower crop productivity. One of the factors affecting plant productivity is the use of qualified planting materials. One of the efforts to provide qualified seedlings can be done by using fast rooting stem cuttings. [4] stated that 10 cm stem cuttings are the optimal size for guava root induction with the success of rooted cuttings of 82.8%.

The use of stem cuttings is more efficient as compared to other methods because the availability of seedlings can be obtained in large quantities, fast growing and can be done as long as the trees for cutting materials are available. [5] states that cultivars Paluma, Pedro Sato, Cortibel 1 and Cortibel 6 are suitable
guava cultivars for propagation as shown of the percentage of rooted cuttings of 92%, 75%, 75%, and 79%.

The growth of roots and shoots of guava stem cuttings can be stimulated by using synthetic or natural growth regulators. Synthetic growth regulators can be Indol Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) or commercial products such as Rootone-F and Root-up. [6] stated that the use of 500 ppm IBA for soaking crystal guava stem cuttings for 120 minutes resulted in 76.7% root cuttings as compared to the control treatment of 65%. The disadvantages of synthetic growth regulators are due to its expensive price and the rare availability. Therefore, the growth regulator which cheaper and easier to obtain is needed as an alternative.

One of the natural product that contain growth regulators is shallot. Shallot contain the hormones auxin and gibberellin which play a role in cell elongation and division [7]. Apart from containing auxin and gibberellin, shallot bulbs also contain vitamin B1 (thiamin). Vitamin B1 has an important role in converting carbohydrates into energy in plant metabolism. In the process of root initiation, plants need energy in the form of nitrogen, glucose and other compounds in sufficient quantities to accelerate root growth [8]. Therefore, application of shallot extract on guava shoot cuttings is expected to spur root growth to be faster.

Plant growth regulators should be applied at the appropriate concentration. Excessive plant growth concentrations results in stunted plants, as well as if it is too small, hormones have no effect on root growth. [9] states that growth regulators can work effectively in influencing plant physiological processes at the right concentration.

The use of shallots as a growth regulator has been tested in several types of plants. [10] revealed that the use of natural growth regulators originating from shallots with a concentration of 1.5% and 2% was able to grow the best agarwood seeds, as indicated by the parameters of leaf number, leaf area, stem circumference, shoot height, dry and fresh weight. Meanwhile, [11] states that application of shallot extract with a concentration of 1.5% shows the best results on root length growth on Gardenia jasminoides Ellis cuttings. In addition, [12] in his research on agarwood cuttings, showed that the shallot concentration of 1.0% was the optimal concentration indicated by the percentage of viable cuttings of 80%. This study illustrates that each plant requires a different concentration of growth regulators. Therefore, research is needed to determine the effect of shallot extract concentration (Allium cepa L. var aggregatum) on the growth of crystal guava cuttings in order to obtain the optimum shallot extract concentration.

2. Materials and Methods
This research was conducted using a single factor experimental method compiled in a completely randomized design (CRD). The treatments tested were the concentration of shallot extract consisting of 1% fresh shallot extract, 1% macerated shallot extract, 2% macerated shallot extract, 3% macerated shallot extract, IBA with a concentration of 500 ppm as control and without growth regulators as control. Each treatment was repeated 4 times, each replication consists of 3 samples and 1 reserve.

2.1. Preparation of growth regulators
2.1.1. IBA (Indole Butyric Acid). 500 ppm IBA was prepared by dissolving 0.05 gram of IBA in a measuring flask with a few drops of 96 percent ethanol. Furthermore, distilled water was added to the flask so that the volume of the solution became 100 ml

2.1.2. Fresh Shallot Extract. Preparation of fresh shallot extract is carried out by blending the shallots and filtering so that 100% fresh shallot extract is obtained.

2.1.3. Macerated Shallot Extract. The maceration method is made by washing the shallot bulbs, then cutting them into pieces and then drying them in the air without being exposed to sunlight. Drying in this way, aims to obtain good quality plant material. The dried tuber pieces are mashed using a blender to obtain the powder (simplicia) for extraction. 200 grams of simplicia powder is macerated with a
solution of 600 ml of 96% methanol (ratio of powder to solvent 1:3). Then the solution was soaked for 24 hours, stirred occasionally, and filtered using a funnel coated with filter paper to get the filtrate, then the pulp obtained is macerated again 3 times so that the solution is almost colorless. The total methanol used was 1.8 L. The filtrate was evaporated with a rotary evaporator at 50°C to obtain a viscous extract which used as a 100% macerated stock solution [11]. The concentration of macerated shallot extract used for this study were 1%, 2% and 3%. The concentration was prepared by diluting the 100% macerated shallot extract with sterile distilled water. The choice of 96% methanol solvent was used because it is cheaper for the main solvent, volatile and can absorb secondary metabolites from simplicia [11].

2.2. Preparation of Shading house and Planting Media
The lid was made with a width of 1 meter, a length of 3 meters and a height of 0.6 meters. The moisture of the rooting medium is maintained by using a transparent plastic cover and a shade of 60% paranet on the top of the lid. Watering was carried out using a mist irrigation system.

The planting medium used for rooting was husk charcoal. This is consistent with the research of [13] which stated that husk charcoal produced the highest percentage of rooted cuttings of 56% in crystal guava cuttings. The husk charcoal was first filtered using a wire net so that no other impurities enter the husk [13]. Then the husk charcoal was dried and put in a nursery pot (poly bag).

2.3. Preparation of Planting Materials (Cuttings)
Crystal guava plant cuttings were selected from parent trees over 3 years old. Guava cuttings used were stem cuttings using young twigs or branches of plants (± 0.5 cm in diameter) and green. The branches were cut into two parts (the young stem at the base and the middle) with a length of 10 cm for each cut [4]. Furthermore, 2 leaves were left at the top of the cuttings and reduce the leaf area by cutting the leaves in half.

2.4. Sterilization and Planting of Cuttings
The base of the cuttings was immersed in 500 ppm IBA, 1% fresh shallot extract, 1%, 2% and 3% macerated shallot extract (according to treatment) for 120 minutes. After soaking, the cuttings were sterilized using a solution of Dithane M45 mixed with water, with a concentration of 5% and sprayed evenly on the cutting material. It was intended that the cutting material was free from fungal attack at the time of planting. The cuttings were planted by plugging them directly into the husk charcoal media in the polybags that prepared previously. The spacing between polybags was 10 cm.

2.5. Parameters Observed and Data Analysis
Observations were made on 3 seed cuttings per treatment, including shoot height, number of leaves and shoot diameter at week 8 to week 12. Meanwhile, the observation of root cuttings percentage, root length, number of roots, root dry weight, leaf area and shoot dry weight were carried out at week 12. The observed data were analyzed using Analysis of Variance (ANOVA) at an error level of 5% and if there were significant differences between the treatments tested, a further test was carried out using Duncan's Multiple Range Test (DMRT).

3. Result and Discussion

3.1. Shoot Height and Diameter
The analysis of variance showed that the concentration of shallot extract had a significant effect on the height and diameter of shoots of crystal guava stem cuttings. The data are presented in table 1. The data on table 1 showed that shoot height in IBA treatment with a concentration of 500 ppm, fresh shallot extract with a concentration of 1%, and macerated shallot extract with a concentration of 2% were significantly higher than those in the treatment of macerated shallot extract with a concentration of 3%, macerated shallot extract with a concentration of 1%, and without plant growth regulators. Treatment of
2% macerated shallot extract resulted in the highest shoot diameter, but it was not significantly different from the treatment of fresh shallot extract with a concentration of 1% and IBA with a concentration of 500 ppm. Shallots (Allium cepa L.) can function as natural growth regulators, because shallot contains the hormones auxin and gibberellin, so they can spur seed growth [14], [15] states that auxin affects the elongation of plant cells.

Table 1. The Effect of Shallot Extract Concentration on the Shoot Height and Diameter of Crystal Guava Cuttings at 12 Twelve Weeks After Planting

| Treatment                                         | Shoot Height (cm) | Shoot Diameter (cm) |
|---------------------------------------------------|-------------------|---------------------|
| Without plant growth regulator                    | 0.32 b            | 0.08 b              |
| Fresh shallot extract 1% concentration             | 1.28 a            | 0.18 ab             |
| Macerated shallot extract concentration of 1%      | 0.44 b            | 0.12 b              |
| Macerated shallot extract concentration of 2%      | 1.75 a            | 0.24 a              |
| Macerated shallot extract concentration 3%         | 0.23 b            | 0.08 b              |
| IBA with a concentration of 500 ppm                | 1.71 a            | 0.16 ab             |

Note: The numbers in the column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

The way auxin works is to affect the elasticity of the cell wall. The cell then grows lengthwise because of the incoming water by osmosis. Cell elongation continues to grow and re-synthesizes cell wall and cytoplasmic material. [16] explained that auxin affects cell elongation through cell wall bending. As the auxin mechanism, namely initiating cell elongation and spurring certain proteins in the cell membrane to pump H+ ions into the cell wall. The H+ ion activates certain enzymes so that they break some of the hydrogen cross-links from the cellulose molecule chains that make up the cell wall. Cells grow and elongate due to water entering by osmosis. In addition to encouraging cell elongation leading to root and stem elongation, the combination of auxin and gibberellin will promote the development of vessel tissue and stimulate cell division in the vessel cambium and thus support the formation of stem diameter. Furthermore [14] added that the gibberellin hormone will stimulate growth on the leaves and stems of plants. The auxin and gibberellin content in 1% fresh shallot extract and 2% macerated shallot extract are thought to play an effective role, such as the IBA in encouraging the growth of height and diameter of shoots of crystal guava cuttings.

3.2. Number of leaves and leaf area

The analysis of variance showed that the concentration of shallot extract had a significant effect on the number leaves and leaf area of crystal guava stem cuttings (Table 2).

Table 2. The Effect of Shallot Extract Concentration on the Number of Leaves and Leaf Area of Crystal Guava Stem Cuttings at Twelve Weeks After Planting

| Treatment                                         | Number of Leaves | Leaf Area (cm²) |
|---------------------------------------------------|------------------|-----------------|
| Without plant growth regulator                    | 0.33 b           | 0.33 b          |
| Fresh shallot extract 1% concentration             | 1.00 ab          | 2.50 a          |
| Macerated shallot extract concentration of 1%      | 0.33 b           | 0.50 b          |
| Macerated shallot extract concentration of 2%      | 1.50 a           | 3.67 a          |
| Macerated shallot extract concentration 3%         | 0.42 b           | 0.33 b          |
| IBA with a concentration of 500 ppm                | 1.42 a           | 4.00 a          |

Note: The numbers in the column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

The data on table 2 showed that the number of leaves in the 2% macerated shallot extract treatment and 500 ppm concentration of IBA was significantly more than the 3% macerated shallot extract treatment, 1% macerated shallot extract and no treatment, but not significantly different with the
treatment of fresh shallot extract with a concentration of 1%. Research by [10] also reported that the use of natural growth regulators from shallots with a concentration of 2% provided the best seed growth.

The results also showed that the IBA treatment with a concentration of 500 ppm, 2% macerated shallot extract and 1% concentration of fresh shallot extract significantly resulted in a larger leaf area than the 1 and 3% macerated shallot extract treatment, and without treatment. Macerated shallot extract at a concentration of 2% is thought to be the right treatment for the growth and development of leaf area on crystal guava cuttings, as stated in [9] which states that auxin can increase osmotic pressure, increase cell permeability, increase protein synthesis and increase plasticity and cell wall development so as to stimulate cell division which can increase or increase the number and area of leaves.

3.3. The Percentage of Rooted Cuttings and Root Length

The analysis of variance showed that the concentration of shallot extract had a significant effect on the percentage of root cuttings and root length of crystal guava stem cuttings (Table 3).

| Treatment                                      | Percentage of Rooted Cuttings (%) | Root Length (cm) |
|------------------------------------------------|-----------------------------------|-----------------|
| Without plant growth regulator                 | 58.35 b                           | 6.35 b          |
| Fresh shallot extract 1% concentration         | 83.35 ab                          | 8.16 b          |
| Macerated shallot extract concentration of 1%  | 58.35 b                           | 6.87 b          |
| Macerated shallot extract concentration of 2%  | 91.68 a                           | 12.67 a         |
| Macerated shallot extract concentration 3%     | 58.35 b                           | 7.25 b          |
| IBA with a concentration of 500 ppm            | 91.68 a                           | 13.33 a         |

Note: The numbers in the column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

IBA is generally more effective than IAA in root initiation [17]. The results of the analysis showed that the IBA treatment with a concentration of 500 ppm and a macerated red onion extract with a concentration of 2% resulted in a significantly higher percentage of rooted cuttings and a longer root length than other treatments, except for the 1% fresh shallot extract treatment which produced the same high percentage of rooted cuttings but the root length was markedly shorter (Table 3). As stated by [18], the function of auxin is to stimulate cell enlargement, chromosomal DNA synthesis, and the growth of the longitudinal axis of plants, which are used to stimulate the growth of cuttings or grafts. The emergence of roots in the treatment of 2% macerated shallot extract was due to the macerated shallot extract containing substances thought to be auxins, other vitamins and minerals that can increase the growth of crystal guava cuttings. Vitamin B1 contained in shallot has an important role in converting carbohydrates into energy in plant metabolism. In the process of root initiation, plants need energy in the form of nitrogen, glucose and other compounds in sufficient quantities to accelerate root growth [18], so as to increase the percentage of rooted cuttings and root length growth.

3.4. Number of Primary, Secondary and Tertiary Roots

The results of the analysis of variance in the number of primary, secondary and tertiary roots of guava stem cuttings are presented in table 4. The data on table 4 showed that the concentration of shallot extract treatment was not significantly different to the number of primary, secondary and tertiary roots. Crystal guava cuttings are thought to contain auxin in the plant. In line with [19] which states that the auxin hormone acts as a driving force for root formation and in fact the plant itself produces a hormone called endogenous auxin. However, there was a tendency for the number of primary, secondary and tertiary roots in the IBA treatment with a concentration of 500 ppm, macerated shallot extract with a
concentration of 2% and fresh shallot extract with a concentration of 1% respectively higher than other treatments.

Table 4. The Effect of Shallot Extract Concentration on the Number of Primary, Secondary and Tertiary Roots of Crystal Guava Stems at Twelve Weeks After Planting

| Treatment                                      | Number of each root type |
|------------------------------------------------|--------------------------|
|                                                | Primary | Secondary | Tertiary  |
| Without plant growth regulator                | 4.22 a  | 38.18 a   | 228.7 a   |
| Fresh shallot extract 1% concentration        | 4.85 a  | 39.18 a   | 345.3 a   |
| Macerated shallot extract concentration of 1% | 2.42 a  | 27.90 a   | 316.0 a   |
| Macerated shallot extract concentration of 2% | 5.67 a  | 51.58 a   | 584.6 a   |
| Macerated shallot extract concentration 3%    | 4.25 a  | 37.00 a   | 277.4 a   |
| IBA with a concentration of 500 ppm           | 6.42 a  | 64.53 a   | 645.6 a   |

Note: The numbers in the column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

3.5. Dry Weight of Roots and Shoots

The analysis of variance showed that the concentration of shallot extract significantly affected the dry weight of the roots and shoots of crystal guava stem cuttings (Table 5).

Table 5. The Effect of Shallot Extract Concentration on Dry Weight of Roots and Shoots of Crystal Guava Stem Cuttings at Twelve Weeks After Planting

| Treatment                                      | Dry weight (g) |
|------------------------------------------------|----------------|
|                                                | Root | Shoot     |
| Without plant growth regulator                | 0.07 b  | 0.008 b |
| Fresh shallot extract 1% concentration        | 0.13 ab | 0.044 ab |
| Macerated shallot extract concentration of 1% | 0.08 b  | 0.008 b |
| Macerated shallot extract concentration of 2% | 0.22 a  | 0.069 a |
| Macerated shallot extract concentration 3%    | 0.12 ab | 0.011 b |
| IBA with a concentration of 500 ppm           | 0.21 a  | 0.058 a |

Note: The numbers in the column followed by the same letter show no significant difference based on the DMRT test at the 5% level.

The results of analysis of the dry weight of roots and shoots showed that the treatment of macerated shallot extract with a concentration of 2% and IBA treatment with a concentration of 500 ppm resulted in significantly higher dry weight of roots and shoots than other treatments except for the treatment of fresh shallot extract with a concentration of 1% which was not significantly different, and the treatment of macerated shallot extract with a concentration of 3% dry weight of the roots was the same. Macerated shallot extract with a concentration of 2% produced the best roots in crystal guava stem cuttings. As stated by [20] that root dry weight is the accumulation of assimilate products throughout plant growth. Research by [10] supported that the use of natural shallot growth regulators with a concentration of 2% could provide the best seed growth. Shallot bulbs contain growth regulating substances, namely auxin which is useful for stimulating root growth and Vitamin B (thiamin) which is useful in the process of converting carbohydrates into energy in plant metabolism. The treatment of macerated shallot extract with a concentration of 2% and IBA with a concentration of 500 ppm accelerated the growth of roots which could supply food for the process of shoot growth on cuttings. This is in accordance with [21]...
statement that the root cuttings that are formed are an important factor because the roots can absorb nutrients contained in the soil and encourage their survival.

4. Conclusion
The 2% macerated shallot extract concentration was able to grow roots and shoots with the same criteria as the IBA concentration of 500 ppm on crystal guava stem cuttings.

References
[1] Indonesian Tropical Fruit Research Institute 2008 Agricultural Research and Development Newsletter 6 17-18.
[2] Wijaya, Y P Fery, Y Bachtir, and R Ariandini 2010 Utilization of Red Guava (Guajava) as Basic Ingredient of Highly Nutritious Steamed Cakes University of Malang : Malang.
[3] BPS 2017 Indonesian Annual Fruit and Vegetable Plant Statistics
[4] Gautam, N N, K. Singh, B Singh, S Seal, A Goel, and V L Goel 2010 AJCS 9 666-669.
[5] Altoo, J A, C S Marinho, M I da Costa Terra, and A J C de Carvalho 2011 Bragantia 4 801-809.
[6] Heriandi, S 2015 Treatment of Concentration and Soaking Time of IBA Hormone (Indole Butyric Acid) Against The Growth of Soft Stem Cuttings of Crystal Guava (Psidium guajava L.) Thesis: Bengkulu State University.
[7] Supriati, Y and Herliana, E 2010 Planting 15 Organic Vegetables in Pot Jakarta : Self-help spreader.
[8] Hartmann, H T, Kester D E, Davies F T, and Geneve R L 1997 Plan Propagation Principles and Practices New Jersey: Prntice Hall Inc.
[9] Abidin, Z 1985 Knowledge Basics About Growth Regulators Space: Bandung.
[10] Siregar, A P, Zuhry E, and Sampoerna 2015 Growth of Gaharu Seeds (Aquilaria malaccensis) by Provision of Growth Regulatory Substances from Onions.
[11] Roni, A 2017 Effect of Red Onion Extract (Allium cepa L) on Root Growth of Gardenia jasminoides Ellis Cuttings and Contribution to Vegetative Propagation Materials
[12] Muswita 2011 The Effect of Red Onion (Allium cepa L.) Concentration on the Growth of Gaharu Cuttings (Aquilaria malaccensis OKEN) Jambi University 13 0852-8349
[13] Jalal A, Supanjanj S, and Sulistyyo, B 2017 Propagation of crystal guava through soft stem cuttings on various rooting media.
[14] Marfiran M, Ratnasari E, and Rahayu Y S 2014 Lantern Journal Bio3 1 73–76
[15] Rusmini, D 2011 Litri's Journal 17 3.
[16] Yunita R, 2011. The Effect of Giving Cow Urine, Coconut Water and Rootone F on Growth of Passion Fruit (Passiflora edulis var. Flavicarpa)
[17] Litwack G 2005 Plant Hormones Vitamins and Hormones Advances in Reasearch and Applications Elseveir Academic Press : Oxford 544.
[18] Dewi, I, R 2008 Role and Phytohormones for Plant Growth Padjadjaran University : Bandung
[19] Prastowo N H, M R James, G E S Manurung, E Nugroho, J M Tukan, and F Harun 2006 World Agroforestry Center (ICRAF) and Winrock International Bogor 100
[20] Gardner F P, Pearce R B and Mitchell R L 2017 Physiology of Crop Plants Oxford: Scientific Publishers
[21] Auri, A and P A Dimara 2016 Tropical Silviculture Journal 2 133-136.