The effect of NAA and coconut water combination on garlic
(*Allium sativum* L.) tissue culture

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**Abstract.** Tissue culture techniques can increase the number of garlic seedlings. The purpose of this research is to determine the effect of NAA and coconut water in increasing the number of garlic seeds. This research used a Completely Randomized Design of two factors. The treatment used is NAA with concentrations of 0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm, and coconut water concentrations of 0%, 10%, 20%. The variables observed were shoot emergence time, root emergence time, number of shoots, number of roots, number of leaves, shoot height, root length, and number of plantlets. The results showed that the addition of coconut water 20% without the addition of NAA in 1 bulb can produce 3.33 planlets and the results of explant propagation in 1 bulb can produce the number of shoots as many as 15.33 shoots. Giving coconut water with concentrations of 10% and 20% can increase the number of leaves, shoot height, and some plantlets. The concentration of NAA 0.5 ppm can accelerate the root emergence time on garlic explant.

1. **Introduction**

Garlic is a spice plant that has high commercial value. One of the local garlic that is much in demand by the public is the *Tawangmangu baru* variety garlic. This is because this *Tawangmangu baru* variety has a strong aroma. *Tawangmangu baru* garlic is known for its strong taste, good growth, and strong stems [1]. Therefore, it is necessary to increase the number of garlic seedlings of *Tawangmangu baru* varieties.

Garlic cultivation through tissue culture technique can produce more seeds in one bulb than conventional planting. Propagation through tissue culture techniques is also known to produce plant seeds in a relatively fast time. Tissue culture techniques are expected to improve the quantity and quality of garlic seedlings. Tissue culture techniques have several advantages over traditional propagation methods, including producing mature plants relatively quickly, plants produced are the result of regeneration of genetically modified cells, efficient in land use [2]. Tissue culture provides all the nutrients, energy, and water needed by plants or explant growth through culture media [3].

One of the factors that affect the success of the growth of garlic explants is PGR (Plant Growth Regulatory) contained in the culture media. Growth regulators that are important for the growth of garlic explants are from the auxin and cytokinin groups. The main use of auxin is to increase the percentage of roots, improve the quality parameters of the number of adventitious roots, the symmetry
of the root system, and the ratio of roots [4]. Cytokinins have a role in the process of cell division, which also functions to regulate the growth of leaves, flowers, fruit, and stimulate the formation of roots and stems. Therefore, if auxin and cytokinin are combined, it can make plant growth effective. This research used NAA from auxin and coconut water from the cytokinin group. Coconut water is traditionally used as a growth supplement in plant tissue culture [5]. Naphthalene Acetic Acid (NAA) is a synthetic auxin that is often added in the growing media because it has more stable properties than other types of auxin [6].

2. Materials and methods
The research was conducted in September 2020 – March 2021 located at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta. The research method used was a two-factor Completely Randomized Design (CRD) with twelve treatment combinations and three replications. The first factor is the concentration of NAA which consists of 4 levels, namely 0 ppm, 0.5 ppm, 1 ppm, and 1.5 ppm. The second factor is the concentration of coconut water 0%, 10%, and 20%. The variables observed were shoot emergence time, root emergence time, number of shoots, number of roots, number of leaves, shoot height, root length, and number of plantlets. The observed data were analyzed for their diversity using the F test at the 5% level and if it was significantly different it was followed by a DMRT of 5% level.

3. Results and discussion

3.1. Shoot emergence time

The time of shoot emergence is one of the important indicators in the multiplication of tissue culture. Efforts to multiply plants can be done quickly when the time of shoot emergence is also faster. The shoot emergence time can be seen when the appearance of the shoot is in the form of lumps with white color and then grows to green color at the top of the explant.

| Coconut water | NAA 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
|---------------|-----------|---------|-------|---------|
| 0%            | 3.33a     | 3.67a   | 3.33a | 4.33a   |
| 10%           | 3.00a     | 4.00a   | 3.67a | 4.00a   |
| 20%           | 3.67a     | 3.33a   | 3.67a | 4.00a   |

3.2. Root emergence time

Roots become one of the important factors in plant growth. The function of the root as part of the plant is to absorb nutrients and nutrients contained in the planting media. Calculation when the emergence of the root is characterized by the presence of white protrusions at the bottom of the explant that grows elongated with a length of approximately 2 mm. The time of root growth is an important factor, plants will be easier in absorbing nutrients contained in cultural media [9]. The addition of a single factor of coconut water and the interaction between NAA and coconut water has no significant effect on the
time of emergence of the root. Cytokinin plays a role in spurring shoot growth but inhibiting root growth [10].

Table 2. Effect of NAA and coconut water on roots emergence time.

| Treatment | Root emergence time |
|-----------|---------------------|
| NAA 0 ppm | 5.89a               |
| NAA 0.5 ppm | 5.56a            |
| NAA 1 ppm | 7.11ab             |
| NAA 1.5 ppm | 7.78b            |

Based on the F test results that can be seen in Table 2, the single factor of NAA has a significant effect on the time of root emergence. The addition of 0 ppm and 0.5 ppm NAA was significantly different from the media treated with 1.5 ppm NAA. MS media added with 1.5 ppm NAA concentration can slow down the time of root emergence, roots can form after 7.78 days after planting. It is thought to be due to the right concentration of NAA that can accelerate the emergence of roots, but if the NAA is too high it causes delays in the emergence of roots. The addition of NAA at high doses leads to loss of root stimulatory effects such as reduced primary root lengthening and inhibition of lateral root formation [11]. The 0.5 ppm NAA treatment had no significant effect on the treatment without NAA, it can be seen in Table 2 that the time of root emergence is followed by the same letter.

3.3. Number of shoots

The number of shoots becomes one of the important parameters in the cultivation of tissue culture. The results of the various analyses using the F test with a confidence level of 5% did not show a significant difference.

Table 3. Effect of NAA and coconut water on the number of shoots.

| Coconut water | NAA          |
|---------------|--------------|
|               | 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
| 0%            | 2.42a   | 3.67a   | 1.83a  | 2.42a   |
| 10%           | 3.25a   | 2.67a   | 2.17a  | 3.67a   |
| 20%           | 3.42a   | 3.83a   | 3.33a  | 3.08a   |

The number of shoots in this research was 1.83-3.83 shoots (Table 3). The highest number of shoots formed was 3.83 shoots. It means that in 1 garlic bulb that has been propagated becomes four. On average in each replication, it can produce 15.33 shoots. The least shoots formed in this study were 1.83 shoots which means that on average, each replication in 1 bottle produced as many as 7.33 shoots. This is because each plant has a different response to its growth on the growth regulator that has been given. Explant conditions affect the growth and success of culture, namely genetic factors, types of explants, size, age, and physiological phase of tissues used as explants [12]. In absorbing exogenous cytokinins not all plants can respond to the addition of shoots because plants have sufficient endogenous cytokine content [13].

3.4. Number of roots

Table 4. Effect of NAA and coconut water on the number of roots.

| Coconut water | NAA          |
|---------------|--------------|
|               | 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
| 0%            | 7.42a   | 6.33a   | 5.25a  | 11.5a   |
| 10%           | 8.25a   | 5.75a   | 10a    | 8.33a   |
| 20%           | 4.42a   | 12.9a   | 9.12a  | 8.12a   |
Plant growth is influenced by the number of roots formed from a plant. The growing number of roots can optimize the absorption of nutrients from the medium to plants and plant growth is expected to be more optimal. The addition of NAA and coconut water in MS media has not been significant, both single factors, and interactions. The results of this research can be seen in Table 4 that the number of roots formed ranged from 4.42-12.9 roots. The average number of roots of each treatment is 8.13. This is because the absorption of regulatory substances to grow each explant varies and the time it takes for plants to spur root growth. To form a rooting plant takes longer for NAA because it has translocation and slow activity [14]. Different cytokinins and auxins can be responded to by different plants [15].

3.5. Number of leaves
Leaves are an important organ in plants because leaves can support plants to perform photosynthesis. Based on the F test at a rate of 5%, the single factor of coconut water has a significant effect. Giving 10% and 20% coconut water to the media had a significant effect compared to the media without the addition of coconut water (Figure 1). Giving coconut water a concentration of 200 ml / l produces the most optimal number of leaves in garlic plant growth [16].

Table 5. Effect of NAA and coconut water on the number of leaves.

| Treatment          | Number of leaves |
|--------------------|-----------------|
| Coconut water 0%   | 2.15a           |
| Coconut water 10%  | 3.46b           |
| Coconut water 20%  | 3.78b           |

Based on the results that can be seen in Table 5, the number of garlic leaves on MS media added with 20% coconut water with various concentrations of NAA showed the highest results, it can produce 3.78 leaves. This is due to the complete content of growth regulators in coconut water that can provide optimal results on the number of leaves. Coconut water can increase the number of leaves because young coconut water has a high content of cytokinin and nitrogen 43 g/l [17]. Tubers grown at higher concentrations of cytokines combined with cytokinin or organic auxin produce a greater number of leaves [18]. The addition of low NAA produced the largest number of leaves [19].

![Figure 1. The number of leaves on the treatment of A (N0A0) and B (N0.5A2).](image)

3.6. Shoot height
One of the important observation variables in this study is shoot height. Higher shoots indicate good growth. The addition of coconut water and the interaction between NAA and coconut water in the MS
media have a significant difference in the height of garlic explant shoots. The addition of coconut water with a concentration of 10% and 20% shows a noticeable difference compared to the treatment without the addition of coconut water in the media MS. Coconut water can be used as an organic additive for plants in the lengthening/regeneration of shoots [20].

Table 6. Effect of NAA and coconut water on shoot height.

| Coconut water | NAA          |
|---------------|--------------|
|               | 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
| 0%            | 22.5cd | 12.2ab  | 11.3a  | 11a     |
| 10%           | 16.7abcd | 21.33bcd | 22.63cd | 13.5abc |
| 20%           | 15.93abcd | 21.93cd  | 24.57d  | 20.93bcd |

Based on the results of test F that can be seen in Table 6, the addition of NAA 0 ppm accompanied by the addition of coconut water up to 20% showed no difference from the treatment of NAA 0.5 ppm, NAA 1 ppm, NAA 0.5 ppm followed by the addition of coconut water 10% and 20%. However, NAA 1 ppm + coconut water 20% showed higher crop yields of 24.57 cm. The addition of NAA 1.5 ppm + coconut water 0% in the media shows a lower crop height yield of 11 cm. The data showed that almost all treatments that were not accompanied by the addition of coconut water showed lower results. Coconut water contains substances, namely carbohydrates, vitamins, cytokines, and a little auxin that are useful for plant growth [21].

The addition of coconut water 20% followed by an increase in NAA to a concentration of 1 ppm tends to produce a good height of plants, but when the NAA concentration is raised to 1.5 ppm causes the height of the plant to drop. The increase in NAA used inhibits the height of plants, the addition of auxin with low concentrations can encourage enlargement and extension of cells [22].

3.7. Root length
The length of the roots can affect the growth of plants, as it will affect the absorption of nutrients useful for plant growth. The length of garlic explant root in Table 7 ranges from 12-22.37 cm. The average root length in each treatment is 17.8 cm. The results of the F test analysis showed that the addition of NAA and coconut water with various concentrations did not have a significant influence on the length of garlic root crops. The addition of exogenous auxin in plants causes root lengthening is hampered because plants have a high content of endogenous auxin [23].

Table 7. Effect of NAA and coconut water on root length.

| Coconut water | NAA          |
|---------------|--------------|
|               | 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
| 0%            | 21.3a  | 22.37a  | 12a   | 15.6a   |
| 10%           | 18.43a | 20.67a  | 12.6a  | 15.16a  |
| 20%           | 13.23a | 22.17a  | 21.77a | 18.27a  |

3.8. Number of planlets
Planlet is a small plant grown aseptically in tissue culture techniques. It already has complete plant parts such as stems, roots, and leaves.

Table 8. Effect of NAA and coconut water on the number of planlets.

| Coconut water | NAA          |
|---------------|--------------|
|               | 0 ppm | 0.5 ppm | 1 ppm | 1.5 ppm |
| 0%            | 1a     | 2abc   | 2abc  | 2.33abc |
| 10%           | 1.67ab | 2.67bcd | 2.33abc | 4d      |
| 20%           | 3.33cd | 3.33cd  | 2.67bcd | 2.33abc |
The results of the F test showed that the treatment of single factor coconut water and interaction between NAA and coconut water had a significant effect on the number of planlets. Based on Table 8 we can see that control treatment significantly different from NAA treatment 0 ppm + coconut water 20%, NAA 0.5 ppm + coconut water 10%, NAA 0.5 ppm + coconut water 20%, and NAA 1 ppm + coconut water 20%, but the higher number of planlets found in NAA treatment 1.5 ppm + coconut water 10% (Figure 2). The higher number of plantlets was shown in the treatment of 1.5 ppm NAA + 10% coconut water, which can produce 4 plantlets, but the recommended treatment is 0 ppm NAA treatment + 20% coconut water which can produce 3.33 plantlets and shows not significant results with 1.5 ppm NAA + 10% coconut water treatment. Treatment of NAA 0 ppm + 20% coconut water is recommended because it can reduce production costs in using NAA. MS media which was not added with NAA and coconut water is only able to produce 1 planlet. This indicates that the addition of auxins and exogenous cytokinins can increase the number of planlets. The application of cytokinins and exogenous auxin is important in garlic tissue culture to stimulate various plant developments and physiological and induce callus formation [24].

The addition of coconut water 10% followed by an increase in NAA concentration up to 1.5 ppm produced the best response to a greater number of planlets. Coconut water as a growing regulatory substance also has an auxin content that can stimulate root growth which will also lead to the growth of shoots and fruits [25]. Auxin has a major role in coordinating many growth and behavioral processes and is essential for the development of plant bodies [26].

4. Conclusion
The result obtained in the present research is that the addition of coconut water 20% without the addition of NAA in 1 bulb can produce 3.33 planlets and the results of explant propagation in 1 bulb can produce the number of shoots as many as 15.33 shoots. Giving coconut water with concentrations of 10% and 20% can increase the number of leaves, shoot height, and the number of planlets. The concentration of NAA 0.5 ppm can accelerate the root emergence time on garlic explant.

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