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In vitro micropropagation of Raja Bulu banana in medium supplemented with coconut water and NAA

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Abstract. Raja Bulu banana is one of popular tropical fruit in Indonesia. Increasing production of Raja Bulu banana can be done by in vitro culture with addition coconut water and NAA. This research was conducted in April-November 2017 at Plant Physiology and Biotechnology Laboratory Faculty of Agriculture, Sebelas Maret University. The experimental design was Completely Randomized Design (CRD) two factors: coconut water (K) with concentration K1 = 50 ml l⁻¹, K2 = 100 ml l⁻¹, K3 = 150 ml l⁻¹, and NAA (A) with concentration A0 = 0 ppm; A1 = 0.5 ppm; A2 = 1 ppm; A3 = 1.5 ppm. The result showed that combination of coconut water at 50 ml l⁻¹ and NAA 0.5 ppm resulted in highest number of shoots (5.6 shoots). Medium supplemented with coconut water 100 ml l⁻¹ and NAA 0 ppm resulted in highest number of leaves (8 leaves). In the medium treated with NAA 1.5 ppm, shoots appeared after six days of planting and yielded the highest number of roots (11.55 roots). Coconut water increased the shoot height at concentrations of 50 ml l⁻¹, accelerated root formation at concentrations of 100 ml l⁻¹, and increased number of roots at concentrations of 50 ml l⁻¹.

1. Introduction

Banana is a very popular tropical fruit commodity, has higher nutrient content compared to other fruits. The main contents in banana are water, carbohydrates, vitamin A, thyanin, vitamin B2 and vitamin C [1]. One of the most popular bananas in Indonesia namely Raja Bulu.

Production of good-quality bananas is experiencing obstacle since it is only planted as garden plants and since the land area to plant bananas is narrow. In order to increase the banana production, in vitro micropropagation can be done by supplementing medium with coconut water and NAA. The most important plant growth regulators are auxin and cytokine. Combination of auxin and cytokine contributes to improve explant regeneration.

Plant growth regulator (PGR) from the auxin group of NAA and coconut water contains PGR from cytokines. This research was conducted in purpose to increase the production of banana by tissue culture techniques using a combination of PGR of young coconut water and NAA, and to investigate the best combination of coconut water concentration and NAA to support the growth of banana.
2. Methods
This research was carried out at Plant Physiology and Biotechnology Laboratory of Faculty of Agriculture, Sebelas Maret University from April to November 2017. Completely Randomized Design (CRD) with 2 factors, namely coconut water concentration and NAA concentration. Coconut water concentration factor consists of 3 levels; 50 ml l\(^{-1}\), 100 ml l\(^{-1}\), and 150 ml l\(^{-1}\). NAA concentration factor consists of 4 levels; 0 ppm, 0.5 ppm, 1 ppm, and 1.5 ppm were used in this study. There were 12 treatments combination with each treatment repeated three times, in total there were 36 experimental units. The observed variables are first shoot emerged, the number of shoots, shoot height, first root emerged, number of root, root length, first emergence of leaf and number of leaf. The results were analysed using Analysis of Variant followed with Duncan Multiple Range Test (DMRT) at 95% confidence level.

3. Result and discussion
3.1. General condition
The growth medium was Murashige and Skoog (MS) by adding Fe-EDTA solution, MS vitamins, and agar. Acidity was regulated by using NaOH or HCl to pH ranging from 5.8 to 6.2 with the addition of coconut water concentrations of 50 ml l\(^{-1}\), 100 ml l\(^{-1}\), 150 ml l\(^{-1}\) and various NAA concentrations of 0 ppm; 0.5 ppm; 1 ppm; 1.5 ppm. According to previous study [2] in tissue culture techniques, the existence of growth regulator substances has a very real effect. Explants used for subculture derived from planted buds for tissue culture. A protocol explains that the in vitro shoot subculture is done by cutting small buds that each part contains at least one bud [3].

3.2. Time of shoots emergence
The emergence of shoots is marked by the presence of bulges on explant allegedly a buddy candidate. The results of variance analysis showed coconut water and NAA did not give a significant effect to the time of shoot emergence (table 1). This is indicated by the results of analysis which states that there is no interaction between the two factors of coconut water and NAA.

| NAA (ppm) | Time of shoot emergence (DAP) |
|-----------|-------------------------------|
| 0         | 6.00 a                        |
| 0.5       | 8.66 ab                       |
| 1         | 9.66 b                        |
| 1.5       | 6.11 a                        |

Provision of various concentrations of coconut water has no significant effect on the time of buds. This is in accordance with the reported study before [4] that the addition of coconut water with high concentration can inhibit the emergence of buds. However, NAA giving to the study had a significant effect on the time of shoot emergence.

The addition of NAA 0 ppm resulted with the fastest shoot emerging time but not significantly different with the highest NAA addition of 1.5 ppm. [5] stated that the addition of higher NAA in MS medium gave significant effect on the time of shoots emergence.

3.3. Shoot height
The addition of coconut water and NAA did not give significant result to shoot height, nor did NAA (Table 2). However, the application of coconut water gives a significant effect on shoot height in accordance with the statement of [6] that coconut water as an organic nutrient on culture medium gives significant result to the growth and shoot formation.
Table 2. Effect of coconut water on shoot height of Raja Bulu banana

| Coconut water (ml l⁻¹) | Shoot height (cm) |
|------------------------|-------------------|
| 50                     | 10.89 b           |
| 100                    | 9.01 ab           |
| 150                    | 7.66 a            |

Increasing the concentration of coconut water can inhibit the growth of the shoot’s height. This is because coconut water as ZPT (growth regulator substances) has gibberellins, auxins, and cytokines content. The hormone content reduces as fruit matures. Coconut water contains hormones auxins and cytokines [7]. Coconut water also contains phenolic compounds in the form of benzoic acid that can inhibit growth.

3.4. Number of shoots
Combination of coconut water 50 ml l⁻¹ and NAA 0.5 ppm resulted the highest number of shoots (Table 3). Both addition of coconut water and NAA did not give significant results, however the interaction of coconut water and NAA had a significant effect.

Table 3. Effect of coconut water and NAA on number of shoots of Raja Bulu banana explant

| Coconut water (ml l⁻¹) | NAA (ppm) | Average |
|------------------------|-----------|---------|
| 50                     | 0         | 2.33ab  | 5.67c   | 2.33ab | 4.67bc | 3.75 |
| 100                    | 0.5       | 5.00bc  | 2.67abc | 3.00abc | 3.33abc | 2.67 |
| 150                    | 1         | 1.33a   | 2.33ab  | 4.33abc | 2.67abc | 2.67 |
| 50                     | 1,5       |         |         |         |         |      |
| 100                    |           |         |         |         |         |      |
| 150                    |           |         |         |         |         |      |

The combination of NAA and coconut water gave a significant effect, the combination was NAA 0.5 ppm and coconut water 7.5%. the addition of coconut water concentration of 50 ml l⁻¹ and NAA 0.5 ppm produces the highest number of shoots, this result is in line with previous report [5]. The addition of a single cytokine or combined with auxin made great results for shoot multiplication [8].

3.5. Number of roots
Result of variance analysis showed that the addition of coconut water and NAA give significant effect to the number of roots. Each concentration has significant difference effect. The concentration of 50 ml l⁻¹ resulted the highest root numbers of 11.91 root (Table 4). This is not in accordance with the previous study that coconut water at high concentrations effectively affects the number of roots [9].
Table 4. Effect of coconut water on number of roots of Raja Bulu banana explant

| Coconut water (ml l⁻¹) | Number of roots |
|------------------------|-----------------|
| 50                     | 11.91 c         |
| 100                    | 9.50 b          |
| 150                    | 6.08 a          |

Supplementing coconut water at low concentrations produced a lot of roots in patchouli plants (Pogostemon cablin) [10]. Table 4 shows that the addition of coconut water concentration gives a linear relationship to the number of roots. Highest number of roots obtained was 11.91 with addition of coconut water of 50ml l⁻¹.

Table 5. Effect of NAA on number of roots of Raja Bulu banana explant

| NAA (ppm) | Number of roots |
|-----------|-----------------|
| 0         | 7.00 a          |
| 0.5       | 8.55 a          |
| 1         | 9.55 ab         |
| 1.5       | 11.55 b         |

Significant effects were shown between the addition of NAA concentrations to the number of roots (Table 5). Higher addition of NAA concentration resulted higher number of roots of Raja Bulu banana. This is in accordance with previous research [11] which stated high concentration of auxin given will lead to permeability of cells in explant so that will encourage the emergence of primordial roots on explant.

The number of roots on the NAA gradually increases with the increase of NAA concentration. This is in accordance with the previous opinion [12] that in its role as a determinant of tissue development, auxin and cytokine should consider their concentration and comparison in the medium. Higher auxin than cytokine causes differentiation leads to root growth.

3.6. First emergence of leaf

Analysis of variance showed no interaction found between coconut water and NAA to the time of first emergence of leaf. Each coconut water treatment and NAA also resulted insignificant effect. The addition of coconut water to MS medium affected the leaf area in potatoes compared to without treatment of coconut water [13]. The result from research in the time of first emergence of leaf from 13 - 20.66 day with an average of 17.50 day.

3.7. Number of leaves

Analysis of variance showed that combination coconut water and NAA has significant effect on the number of leaves (Table 6). Cavendish banana tissue culture using MS medium supplemented with auxin and cytokine gave a significant result on the number of leaves [14].
Table 6. Effect of combination of coconut water and NAA on number of leaf of Raja Bulu banana explant

| Coconut water (ml l⁻¹) | NAA (ppm) | 0  | 0,5 | 1   | 1,5 | Average |
|------------------------|-----------|----|-----|-----|-----|---------|
| 50                     | 3.67ab    | 6.33abc | 4.33ab | 6.67bc | 5.25 |
| 100                    | 8.00c     | 4.00ab  | 3.67ab | 5.00abc | 5.17 |
| 150                    | 3.00a     | 3.33ab  | 6.33abc | 4.33ab | 4.25 |
| Average                | 4.89      | 4.55   | 4.78  | 5.33 (+) |     |

Combination of coconut water and NAA shows a quadratic equation to the number of leaves. It can be informed that supplementing coconut water concentration of 50 ml l⁻¹ and NAA 0 ppm produced the highest number of leaves while supplementing NAA with concentration of 0.5 ppm, 1 ppm and 1.5 ppm indicates fewer leaf number. Supplementing coconut water on MS medium gave significant result to shoot height and number of leaves on olive plant [15].

4. Conclusions
The conclusions of this study are as follows, coconut water 50 ml l⁻¹ increases shoot height and number of roots, while at the concentration of 100 ml l⁻¹ accelerates the first emergence of root. NAA of 0 ppm and 1.5 ppm resulted the same time of first emergence of shoot, while NAA 1.5 ppm produces the highest number of roots. Combination of coconut water of 50 ml l⁻¹ and NAA 0.5 ppm results highest number of shoots, while coconut water of 100 ml l⁻¹ and NAA 0 ppm results highest number of leaves.

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