Effect of Rootone-F concentration on the cutting-growth of three species of coffee (Coffea sp.)

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Abstract. Liberica, Robusta and Excelsa coffee are cross-pollinated so that their vegetative propagation uses cuttings. Plant growth regulators (PGR) such as Rootone-F could accelerate root growth on cuttings. The study aimed to determine the effect of Rootone-F concentration on the growth of one segment cuttings. The study was conducted at the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI) from January to March 2020. The research design was a factorial, Completely Randomized Design, with three replications. The first factor was coffee species, i.e., Robusta (BP 936), Liberica (LIM 1 and LIM 2), and Excelsa, and the second factor was Rootone-F concentrations were 0, 100, 200, 300, 400, and 500 ppm. The characters observed were callus diameter, leaves number, shoots number, and leaf length. The results showed there was an interaction between coffee species and Rootone-F concentration on shoots number and leaf length. Every factor affected callus diameter and leaves number, respectively. Application without Rootone-F (0 ppm) gave a higher value of callus diameter and leaves number of LIM 1. Rootone-F concentration that could increase the shoots number and leaf length was 500 ppm for Lim 1, 400 ppm for Lim 2, 0 ppm for BP 936, and 100 ppm for Excelsa.

Keywords: Excelsa, Liberica, plant growth regulators (PGR), Robusta, vegetative

1. Introduction

Coffee (Coffea spp) is a plantation plant that has been cultivated in Indonesia for a long time. Apart from being a source of income for the people, coffee is the main commodity for exports and a source of foreign exchange earnings [1]. Data from the International Coffee Organization [2] recorded that the value of Indonesian coffee consumption in the 2016-2017 period reached 4.6 million packages equivalent to 60 kg/lb. Coffee beans are widely consumed in the form of drinks. Many entrepreneurs move downstream, namely opening cafes that serve various kinds of beverages made from coffee beans. Arabica and Robusta coffee are the types of coffee that were cultivated widely in Indonesia. However, recently Liberica and Excelsa coffee cultivation has grown. Liberica and Excelsa's market shares are Singapore and Malaysia. The market demand for liberica coffee is quite high; therefore, needed a continuous product supply. In Jambi, the price of Liberica green bean is higher than Robusta and Arabica coffee at IDR 30,000/kg. The price of Excelsa coffee in Malaysia is between Rp. 44,800-51,200/kg [3]. Liberika Meranti 1 (LIM 1) and Liberika Meranti 2 (LIM 2) coffees have a preference
value above 80 with excellent taste quality, and Robusta has a very good taste quality, therefore the 3 coffee clones are feasible to be developed [4].

Robusta, Excelsa, and Liberica are cross-pollinated plants [5, 6, 7]. Therefore, this type of coffee is propagated vegetatively using cuttings. Farmers mostly carry out plant propagation with one-segment cuttings by cutting Orthotropic branches for propagation [8]. The cuttings already have endogenous plant growth regulators (PGR) but are often available in small quantities so that additional sources from outside (exogenous) are needed for callus, root, and shoots growth. Adding PGR to soaking media will increases the cutting’s quality, and decrease the number of seedlings below normal standards [9]. The advantages of the cuttings are simple to prepare, save buds, do not depend on the season, do not limited by compatibility, and are relatively resistant to water shortages [10]. The problem in plant propagation by cuttings is the difficulty of root formation, and efforts to accelerate the formation of roots can be done by using growth regulators (PGR).

Plant growth regulators (PGR) can accelerate shoots and roots growth on one segment cuttings. One of the growth regulators used is auxin. One of auxin is Rootone-F. Rootone-F contains indole butyric acid (IBA) and naphthalene acetic acid (NAA), which are auxin groups. Auxin is a growth substance that stimulates elongation so that it can increase the formation of plant roots and shoots [11]. Research conducted by Kurniawan [12] on Robusta coffee with the addition of 100, 150, and 200 ppm Rootone-F showed that 200 ppm Rootone-F could increase the growth of cuttings. The study aimed to determine the effect of Rootone-F concentration on the growth of cuttings. The purpose of this study was to determine the growth of cuttings of Liberica, Robusta, and Excelsa coffee cuttings at various concentrations Rootone-F (0, 100, 200, 300, 400, and 500 ppm).

2. Material and method
The study was conducted at the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI) from January to March 2020.

The research design was a factorial, which was arranged in Completely Randomized Design, with three replications, there were ten cuttings per unit. The first factor was coffee species, i.e., Robusta (BP 936), Liberika Meranti (LIM 1) and Liberika Meranti 2 (LIM 2), and Excelsa (Figure 1), and the second factor was Rootone-F concentrations i.e., 0, 100, 200, 300, 400, and 500 ppm. The characters observed were callus diameter, leaves number, shoots number, and leaf length.

Planting materials used were cuttings of one segment of Liberica coffee clones LIM 1 and LIM 2; Robusta coffee clone BP36; and Excelsa. Cutting was obtained from the coffee branch aged four months after pruning from Agro Widy Wisata Ilmiah (AWWI). Orthotropic stems 60-70 cm long, cut in to 4-5 one segment cuttings.

![Figure 1](image.png)

**Figure 1.** Cuttings of coffee species A. Lim 1 B. Lim 2 C. BP 936 D. Excelsa

2.1 Rootone-F preparation
This research used chemical Auxin, Rootone-F. Rootone F contains 1-naphthalene-acetamide (NAD) 0.006%, 2-methyl-1-naphthaleneacetic acid (MNAA) 0.333%, 3-methyl-1-naphthalene-acetamide
(MNAD) 0.013%, indole-3-butyric acid (IBA) 0.057% and tetramethyl-thiuram disulfide (Thiram) 4% [13]. Rootone-F preparation was carried out at Laboratory by making a solution of 2000 mg/2 L of Rootone-F, 2 g powder was dissolved with 2 liters water. Rootone-F with concentration 100 ppm, the ratio is 100 ml of concentrated solution / 900 ml of water; Rootone-F 200 ppm, the ratio is 200 ml of concentrated solution / 800 ml of water and so on.

2.2 Cutting preparation

Planting materials used were cuttings of one segment of Liberica coffee clones LIM 1 and LIM 2; Robusta coffee clone BP 936; and Excelsa. Cutting was obtained from the coffee branch aged four months after pruning from Agro Widya Wisata Ilmiah (AWWI). Orthotropic branch 60-70 cm long, cut in to 4-5 one segment cuttings. The cutting material is then placed in a plastic container and placed in an experimental unit covered by a lid so that it does not wither in the greenhouse.

The activity of preparing cuttings is carried out on the same day to avoid evaporation of excessive temperatures. The cuttings were cut, then soaked in Rootone-F solution according to the treatment, namely 0.100, 200, 300, 400, 500 ppm Rootone-F solution for 20 minutes. After soaking with Rootone-F, the cuttings are then planted in beds and labeled (Figure 2). For the control treatment, planting material was planted directly on the beds. Cuttings were taken care of by watering two times per day with the addition of Dithane M-45 1% to overcome fungal attacks and weeding of weeds growing on the media.

![Figure 2. A. Cuttings planted on sand media. B. Seedbed in greenhouse.](image)

2.3 Observation and data collection

The characters observed were callus diameter, leaves number, shoots number, and leaf length (Leaf length was measured from the base to the tip of the stalk on all plant leaves). Data was collected at 12 weeks after planting (WAP).

2.4 Data analysis

The data obtained were analyzed statistically using Analysis of variance (ANOVA). If there was a significant difference between treatments, proceed with Duncan's multiple range test (DMRT) at the 5% level [14]. The data processing was carried out using SPSS 2.1 (Statistical Package for the Social Science) application.

3 Result and discussion

Analysis of variance (ANOVA) (Table 1) showed there were interactions between coffee species and Rootone-F concentration on shoots number and leaf length. Each single factor affected callus diameter and leaves number, respectively.
Table 1. ANOVA of the effect of coffee species and Rootone-F concentration to callus diameter, shoots number, leaves number, and leaf length at 12 weeks after planting (WAP).

| Character      | Coffee species | Rootone-F concentration (ppm) | Coffee species * Rootone-F concentration (ppm) | CV % |
|----------------|----------------|-------------------------------|-----------------------------------------------|------|
| Callus diameter| <.0001*        | 0.03*                         | 0.47**                                        | 21.70|
| Shoots number  | 0.11*          | 0.00*                         | 0.00*                                         | 22.37|
| Leaves number  | 0.00*          | 0.02*                         | 0.78**                                        | 27.10|
| Leaf length    | 0.98**         | 0.00*                         | 0.02*                                         | 30.35|

Note: * and ** significant at 5% and 1% level respectively

3.1. Effect of coffee species on callus diameter and leaves number

The results of Duncan’s significant difference test at 5% level showed LIM 1 had the highest of the callus diameter and leaves number at 12 WAP (Table 2), although not significantly different with LIM 2 and Excelsa. The data shows that the growth of Liberica and Excelsa coffee cuttings is faster than that of Robusta coffee. This could be due to the morphological differences between the three coffee species. Plant type plays a role in utilizing plant cutting. According to Arifin and Nurhayati [15], plant cuttings ability to form roots depend on the species. There are species easily grow roots and while other types possess low rate of root formation. Some types are incapable to grow root, despite being given special treatment.

Table 2. Effect of coffee species on callus diameter and number of leaves at 12 WAP

| Coffee species | Callus diameter (mm) | Leaves number |
|----------------|----------------------|---------------|
| Lim 1          | 2.90 a               | 2.92 a        |
| Lim 2          | 2.59 a               | 2.84 a        |
| BP 936         | 1.49 b               | 1.69 b        |
| Excelsa        | 2.74 a               | 2.90 a        |

Note: The numbers followed by different letters in the same column are significantly different according to DMRT test Level = 5%.

Duncan’s significant difference test result at 5% level showed that without Rootone-F application (0 ppm) gave the highest of the callus diameter and leaves number at 12 WAP (Table 3). Under clonal chamber condition, the heat build-up inside causes the formation of adventitious roots in the basal end [16]. The increase of concentration Rootone-F reduces the callus diameter and leaves number at 12 WAP. High concentration Rootone-F actually reduce leaves number. This is in line with research in Robusta coffee, auxin in particularly NAA above 100 ppm reduces the number of leaves [17]. Based on Liberica coffee research by Erdiansyah [18], increasing the concentration of IBA decreases callus diameter. These data indicated that the influence of exogenous hormones at 12 WAP was decreased. Auxin is a growth substance that stimulates elongation to increase the formation of plant roots and shoots. Indole-3-butyric acid (IBA) and a-naphthalene acetic acid (NAA) are even more effective than synthetic or natural IAA for roots. IBA and NAA are still the most widely used auxins for rooting stem cuttings and rooting micro-cutting produced by tissue culture [16]. Indole-3-acetic acid (IAA) is the main component of auxin [19]. First, auxins are produced in active meristematic tissues, namely shoots, young leaves, and fruit. Then auxin spreads widely throughout the plant body, spreading from top to bottom to the point of root growth through the sieve tube network (phloem) or parenchymal tissue [20].
Table 3. Effect of auxin concentration on callus diameter and leaves number at 12 WAP.

| Rootone-F concentration (ppm) | Callus diameter (mm) | Leaves number |
|-------------------------------|----------------------|---------------|
| 0                            | 2.83<sup>a</sup>     | 3.43<sup>a</sup> |
| 100                           | 2.61<sup>ab</sup>    | 3.03<sup>ab</sup> |
| 200                           | 2.29<sup>bc</sup>    | 2.54<sup>bc</sup> |
| 300                           | 2.41<sup>abc</sup>   | 1.79<sup>c</sup>  |
| 400                            | 2.25<sup>bc</sup>    | 2.27<sup>bc</sup> |
| 500                           | 2.11<sup>c</sup>     | 2.38<sup>bc</sup> |

Note: The numbers followed by different letters in the same column significantly differ according to DMRT test Level = 5%.

3.2. Effect of coffee species and Rootone-F concentration on number of shoot and leaf length

Based on the highest average value on the shoots number and leaf length, concentration Rootone-F that could increase the shoots number and leaf length was 500 ppm for LIM 1, 400 ppm for LIM 2, and 100 ppm for Excelsa (Table 4). While, the Rootone-F application at BP 936 significantly reduces the shoots number and leaf length. This result is different from the other research on Robusta coffee. Rootone-F 100-150 ppm increased root length and growth index [21, 22]. Giving Rootone-F 100 ppm to Manglid plant resulted in the average shoots number (5), root length (8.85 cm), and average root number (6.75) and was significantly different from other treatments [23]. Giving a concentration of Rootone-F 200 ppm increases growth rate on the ability to sprout, root length, and the number of leaves of Jabon (Anthocephalus cadamba) shoot cuttings [24]. The phytohormone auxin is an important regulator that sustains the growth and development of the plant. The success of cuttings in coffee is indicated by the emergence of new roots and shoots on cuttings. The emergence of roots and shoots is a process of adaptation and the occurrence of a differentiation process. Most of the differentiation is supported by hormones originating from the plant (endogenous), including auxins and cytokinins, which are translocated from the leaves and shoots to the basal part of the cuttings [16]. The uneven translocation of hormones has a different effect on each plant, for example, giving the effect of bud formation first compared to root formation and vice versa for other types of clones.

Table 4. The average value treatment combination of shoots number and leaf length at 12 weeks after planting (WAP)

| Coffee species | Rootone-F concentration (ppm) | Shoots number | Leaf length |
|---------------|-------------------------------|---------------|-------------|
| LIM 1         | 0                             | 2.30<sup>bdef</sup> | 2.00<sup>bde</sup> |
|               | 100                           | 2.77<sup>bc</sup>     | 1.87<sup>de</sup> |
|               | 200                           | 2.27<sup>bdef</sup>   | 2.27<sup>abde</sup> |
|               | 300                           | 2.47<sup>bdef</sup>   | 1.61<sup>d</sup> |
|               | 400                           | 2.41<sup>bdef</sup>   | 2.31<sup>abde</sup> |
|               | 500                           | 2.61<sup>bdef</sup>   | 2.81<sup>b</sup> |
| LIM 2         | 0                             | 2.55<sup>bdef</sup>   | 3.17<sup>bc</sup> |
|               | 100                           | 2.78<sup>bc</sup>     | 2.19<sup>abde</sup> |
|               | 200                           | 1.61<sup>ef</sup>     | 1.73<sup>d</sup> |
|               | 300                           | 2.71<sup>bc</sup>     | 2.49<sup>bcde</sup> |
|               | 400                           | 3.11<sup>b</sup>      | 2.22<sup>abde</sup> |
|               | 500                           | 1.66<sup>ef</sup>     | 1.53<sup>d</sup> |
| BP 936        | 0                             | 4.00<sup>a</sup>      | 2.82<sup>b</sup> |
|               | 100                           | 1.76<sup>cdef</sup>   | 2.03<sup>bcde</sup> |
|               | 200                           | 1.77<sup>cdef</sup>   | 2.00<sup>bcde</sup> |
4. Conclusion
Application without Rootone-F application (0 ppm) gave higher value of callus diameter and leaves number. LIM 1 had the highest of the callus diameter and leaves number. The increase of concentration Rootone-F reduces the callus diameter and leaves number at 12 WAP. There are several recommendations for vegetative coffee propagation, namely: concentration Rootone-F that could increase the shoots number and leaf length was 500 ppm for Lim 1, 400 ppm for Lim 2, 0 ppm for BP 936, and 100 ppm for Excelsa.

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Contribution Statement
In This paper, Cici Tresniawati, Muhamad Husin, and Luluk Sutji Marhaeni acted as main contributors.

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