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ROOT INDUCTION ON THE SHOOTS OF RUBBER TREE (*Hevea brasiliensis*) CLONE PB 260 THROUGH EX VITRO TECHNIQUE

Induksi Akar pada Eksplan Pucuk Tanaman Karet (*Hevea brasiliensis*) Klon PB 260 Menggunakan Teknik *Ex Vitro*

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ABSTRACT
*Rubber tree (*Hevea brasiliensis*) is a crucial plantation commodity for Indonesia's national development. However, the country's rubber productivity lags behind that of other rubber-producing nations. To address this, propagating rubber plants from superior clones through* ex-vitro *techniques becomes essential.. This study aimed to determine the optimal concentrations of IBA and NAA for root induction in rubber tree shoot explants. A factorial complete randomized design was employed, with the first factor being IBA concentration (0, 100, 200, 300, 400, and 500 ppm) and the second factor being NAA concentration (0, 50, 100, and 150 ppm). The results revealed that the percentage of surviving explants after induction with IBA and NAA did not significantly differ. The rooted explants were obtained using the following treatments: IBA 200 ppm and NAA 0 ppm, IBA 200 ppm and NAA 50 ppm, IBA 300 ppm and NAA ppm, and IBA 400 ppm and NAA 0 ppm. Various concentrations of IBA and NAA did not significantly affect root formation in rubber tree shoot explants.*

Keywords: *Rubber tree; shoots; root induction; IBA ;NAA; ex vitro*

ABSTRAK
Tanaman Karet (*Hevea brasiliensis*) merupakan salah satu komoditas perkebunan penting dan strategis dalam pembangunan nasional. Kendala perkebunan karet di Indonesia yaitu memiliki tingkat produktivitas yang rendah dibandingkan dengan negara penghasil karet lainnya. Produktivitas tanaman karet dapat dipertahankan dengan menggunakan tanaman karet dari klon unggul yang diperbanyak secara *ex vitro*. Tujuan penelitian ini adalah memperoleh konsentrasi IBA dan NAA yang optimal untuk induksi akar pada eksplan pucuk tanaman karet. Rancangan penelitian yang digunakan adalah Rancangan Acak Lengkap Faktorial. Faktor pertama adalah konsentrasi IBA dengan 6 taraf (0, 100, 200, 300, 400 dan 500 ppm). Faktor kedua adalah NAA dengan 4 taraf (0, 50, 100 dan 150 ppm). Hasil menunjukkan persentase hidup eksplan dan persentase berakar setelah diinduksi dengan IBA dan NAA tidak berpengaruh nyata. Pada akhir pengamatan eksplan yang berhasil berakar adalah eksplan dengan perlakuan IBA 200 ppm dan NAA 0 ppm, IBA 200 ppm dan NAA 50 ppm, IBA 300 ppm dan NAA ppm, dan IBA 400 ppm dan NAA 0 ppm.

Kata Kunci: Eksplan pucuk, ex vitro, induksi akar; IBA dan NAA tanaman karet

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INTRODUCTION

Rubber tree (*Hevea brasiliensis*) is a significant plantation crop in Indonesia, providing a crucial source of non-oil and gas income. While 80% of Indonesia's rubber production is exported worldwide, domestic demand for rubber only accounts for 20%. In 2015, rubber exports reached 2.6 million tons valued at US$3.01 billion (BPS 2020). The growing global demand for rubber presents an opportunity for Indonesia to become the largest rubber exporter globally. Despite having the largest rubber plantation area, Indonesia's productivity lags behind other rubber-producing countries. Thailand achieves a production rate of 1,800 kg/ha per year, followed by Vietnam with 1,720 kg/ha and Malaysia with 1,510 kg/ha, while Indonesia's yield is only 1,080 kg/ha (Harahap and Segoro 2018). As rubber is a leading export commodity, it is essential to continue developing and rejuvenating rubber cultivation using superior clones (Marpaung 2017).

The low productivity of Indonesian rubber plantations can be attributed to several factors, including inadequate technology and plantation management, utilization of low-quality or non-superior clones, and the susceptibility of rubber plants to diseases. The use of inferior quality seeds is often a consequence of using generative systems for seed propagation. However, generative plant propagation results in genetic variations from the parent plant (Nuroniah et al. 2018). This study employs clone PB 260, which is known for its superiority in rubber production, fast growth, and resistance to *Colletotrichum corynespora* and *Oidium heveae* (Arif et al. 2016). Currently, superior seeds can be produced through stump grafting, a technique that involves grafting buds from superior rubber seedlings onto the upper and lower stems (Arif et al. 2016).

According to the strategic plan of the Directorate General of Plantation, Ministry of Agriculture Indonesia (2020), the rejuvenation and expansion of rubber plantations program from 2020 to 2025 will require an estimated area of 1.742 million hectares (including rubber plantations). To achieve high crop productivity, it is recommended to plant superior seeds during the rejuvenation and expansion of rubber plantations program. Consequently, the demand for superior seedlings will increase. Therefore, enhancing rubber plant production capacity can be achieved through *ex vitro* propagation techniques using shoot explants.

Ex vitro plant multiplication refers to vegetative propagation carried out under controlled conditions outside the laboratory (Karyanti et al. 2016). The advantages of *ex vitro* techniques lie in their simplicity, cost-effectiveness, and flexibility, as they can be performed at any time of the year (Joni et al. 2015). However, the low survival rate poses a constraint to *ex vitro* breeding, mainly due to the limited root formation in explants. Root induction is essential for nutrient absorption in the soil, which is crucial for the subsequent growth of explants (Putri 2009).

The success of root induction is influenced by the choice of explants (Sudrajat et al. 2018). Therefore, it is necessary to select suitable explants that promote root formation. Shoot explants derived from *ex vitro* techniques offer advantages compared to other types of explant materials. According to Sudrajat et al. (2018), shoot explants from the uppercuts exhibited a root percentage of 65.71%, compared to 33.3% in the middle cut and 33.10% in the lower cut. The induction of roots is influenced by endogenous hormones and food reserves in explants, facilitating cell division for root formation (Pujawati et al. 2017). Shoot explants are obtained by pruning the parent plant, a rejuvenation technique aimed at obtaining physiologically juvenile explants (Kurniaty et al. 2016).

Root formation can be induced using plant growth regulators (PGR) (Sudrajat et al. 2018), which typically contain auxin, a hormone required for root development. The choice of PGR for root formation includes indole butyric acid (IBA), naphthalene acetic acid (NAA), and indole acetic acid (IAA), all belonging to the auxin group (Zhang et al. 2015). The concentration of PGR needed varies for each plant species, as different concentrations yield different effects on growth and development. The optimal concentration of auxin is essential for achieving desired outcomes (Alfiansyah et al. 2015). Admojo (2020) successfully induced rubber plant roots by 54.84% using 500 ppm IBA in seedling explants. In this study, the optimization of IBA and NAA concentrations was conducted to improve the success of root induction in rubber plant shoot explants.

MATERIALS AND METHODS

Location and time

The research was conducted from May to November 2020 at the screen house located in the Biotechnology Laboratory of BRIN, Building 630, BJ Habibie Science and Technology Area, Serpong, South Tangerang.

Materials and tools

The materials used in this study included latosol soil as the planting media, IBA solution, NAA, and shoot explants of rubber plant clones PB 260 that were 6 weeks old and obtained from the plant collection of the Biotechnology Laboratory of BRIN.

Experimental design

The research followed a factorial completely randomized design consisting of two factors. The first factor was IBA with six levels (0, 100, 200, 300, 400, and 500 ppm), and the second factor was NAA with four levels (0, 50, 100, and 150 ppm), resulting in a total of 24 treatment combinations. Each treatment was replicated three times, resulting in 72 experimental units per treatment across the repetitions, with four plants in each unit.

Observation and data analysis

Observations were conducted weekly for 16 weeks. The data collected consisted of pre-treatment data and treatment data. Pre-treatment data included the condition of the mother plant and explants (growth and morphology of the mother plant), chlorophyll content, and auxin content. Chlorophyll content was measured using the Konica Minolta SPAD-502, while the auxin content was analyzed using UV-vis spectrophotometry at a wavelength of 280 nm. The measurement of chlorophyll and auxin content in the leaves was performed on leaves aged 3-8 weeks after cutting. The treatment data included the number of surviving plants, number of leaves, number of rooted plants, number of roots, and root length.

Data analysis was conducted using a Two-way Analysis of Variance (ANOVA) with SPSS 2.0 to determine the effect of the treatment on the observed parameters. If a significant difference was found, the Duncan Multiple Range Test (DMRT) at a 5% significance level was used.

$$Percentage\ of\ survival = \frac{Number\ of\ survive}{Number\ of\ planting} x\ 100$$

$$Percentage\ of\ rooted = \frac{Number\ of\ rooted}{Number\ of\ planting} x\ 100$$

RESULTS AND DISCUSSION

Growth and morphology of shoots on mother plant rubber clone PB 260

The growth of shoots on the mother plant rubber clone PB 260 increased during the first six weeks after pruning. The number of shoots, number of internodes, and number of leaves showed an increasing trend until the fourth week, while other parameters continued to grow until the sixth week (Table 1). After the fourth week, the increase in the

Table 1. Number and average of shoot growth parameters on rubber mother plants for 6 weeks

Parameter	Week-1	Week-2	Week-3	Week-4	Week-5	Week-6
Number of shoots	2.60	2.80	3.10	3.30	3.30	3.30
Height of shoots (cm)	2.15	8.97	9.32	12.83	13.39	15.20
Number of internodes	1.00	3.00	4.00	4.00	4.00	4.00
Number of leaves	1.00	5.00	5.00	7.00	7.00	7.00
Length of leaves (cm)	0.24	5.98	10.24	14.07	14.99	16.14
Width of leaves (cm)	0.05	2.36	4.02	5.25	5.32	6.06

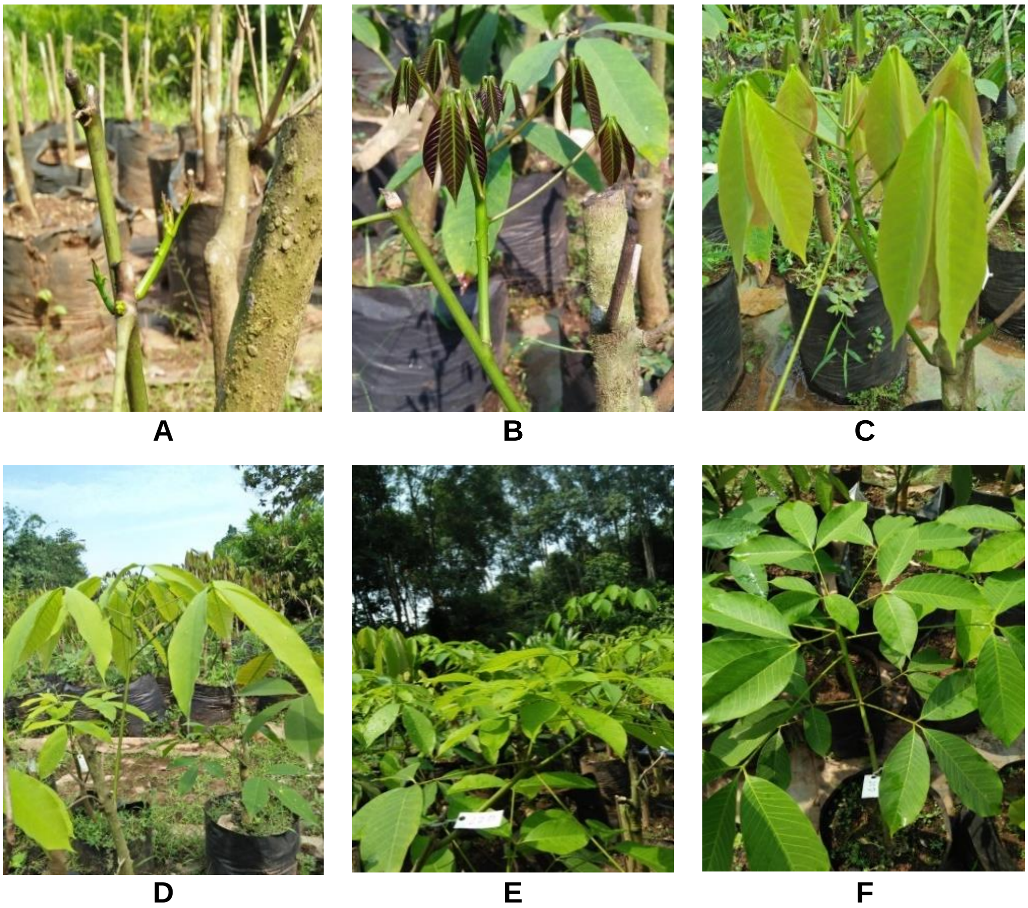

Figure 1. Growth morphology of shoot explants. Shoot explant 1st week after cutting (A), 2nd week explant (B), 3rd week explant (C), 4th week explant (D), 5th week explant (E), and 6th week explant (F)

number of shoots, buds, and leaves stagnated faster compared to the other parameters. Rahayu et al. (2014) suggested that shoots formed simultaneously at the beginning of growth and gradually stopped growing, with growth activity focusing more on shoot elongation. A similar phenomenon was observed in the number of leaves, where the increase in leaf count stopped earlier compared to shoot length. Shoot growth is influenced by the presence of cytokinin and auxin hormones in plant tissue. Auxins induce callus and cell elongation, while cytokinins promote callus proliferation and shoot formation (Rahman et al. 2021).

Shoots are formed after pruning the mother plant rubber clone PB 260. Following pruning, the supply of auxin from the apical shoots ceases, and the roots continue to produce cytokinins, which are transported to the shoots, stimulating shoot growth by increasing cell division (Anggarsari et al. 2017). The purpose of pruning is to obtain physiologically juvenile shoot explants (Kurniaty et al. 2016). Juvenile properties are necessary for explants to induce rooting in plants because roots play a role in nutrient uptake from the soil, which is essential for explant growth (Putri 2009). Juvenile explants exhibit better rooting ability than older explants due to their high cell elongation capacity (Darwo et al. 2018). Juvenile explants can influence root success and determine the speed of root formation and growth process (Kurniaty et al. 2016). Additionally, Atmojo et al. (2013) stated that plants in the juvenile phase are known to have faster and maximum vegetative growth. The juvenile phase is characterized by the formation of the first leaves until before the formation of the first flower on the plant.

The morphology of shoot explants showed changes every week. In the 1st week, the shoot explants had green shoots, copper-red leaves in bud state, and reddish-green stalks. In the 2nd and 3rd weeks, the leaf color changed to brownish green while the stalk remained green. From the 4th to the 6th week, the leaf color changed to dark green, and the

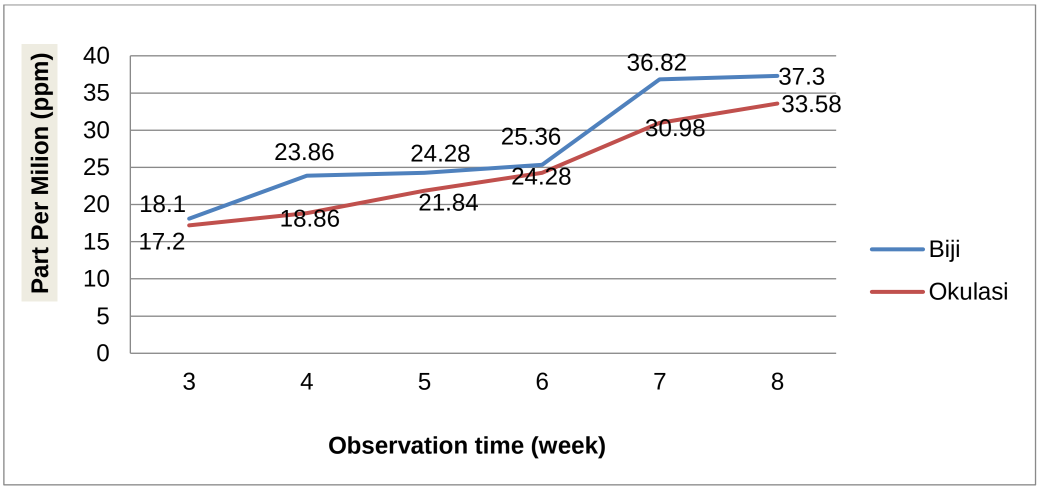

Figure 2. The chlorophyll content in shoot explants from seeds and grafting at 3rd to 8th weeks

leaves were in bloom (Figure 1). These observations are consistent with the findings of Junaidi et al. (2017) that leaf growth can be observed through changes in leaf size and color.

Total content of chlorophyll and auxin in shoot explants

The chlorophyll content in the mother plant from seed and stump increased from the 3rd week to the 8th week after pruning (Figure 2). This is also shown in the growth of leaf color, which shows a change from greenish-red to green color with increasing leaf age. The increase of chlorophyll content that occurs every week is caused by increasing leaf age and leaf growth. The growth of the leaves is marked by color changes that occur; the older the leaf color, the higher the chlorophyll content, since the older leaf color contains higher chlorophyll.

Chlorophyll is the main factor that affects the process of photosynthesis. The function of photosynthesis is to produce carbohydrates needed for plant growth (Ai and Banyo 2011). The chlorophyll content in explants from seeds was higher than in a stump. It is assumed that explants from seeds were more juvenile than a stump, so the photosynthesis process to produce carbohydrates is more optimal. The content of chlorophyll affects the process of photosynthesis. Low levels of chlorophyll will make the photosynthesis process not optimal, so the carbohydrate compounds produced are also not optimal (Pratama and Laily 2015). According to research by Kurniaty et al. (2016), explants with more juvenile characteristics had more carbohydrate content than explants with an older age.

The content of auxin in plants from seeds increased from the 3rd to 4th week after cutting, then decreased from the 5th to 7th week, and increased again at the 8th

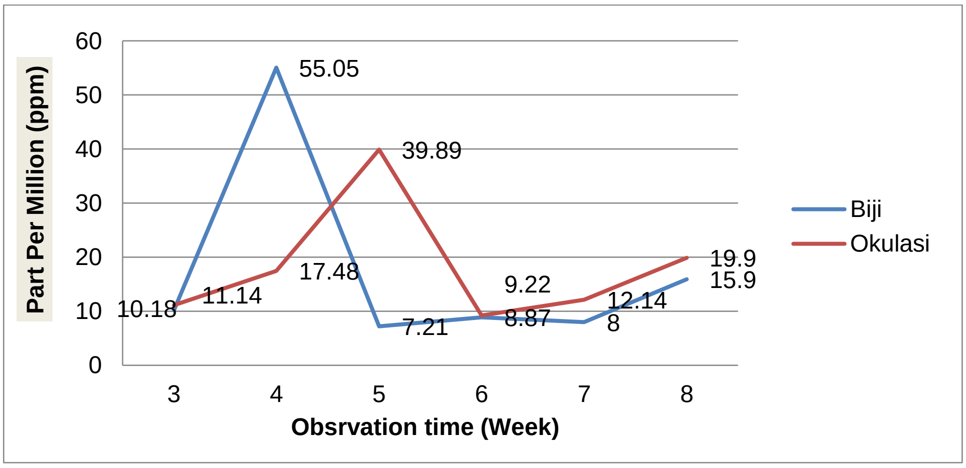

Figure 3. Auxin content in explants of rubber plants from seeds and from the stump at 3rd to 8th weeks

week. While in explants from the stump, it increased from the 3rd to 5th weeks, then decreased from the 6th to 7th weeks, and increased again at the 8th week (Figure 3).

According to the research by Junaidi et al. (2017), the function of auxin content is to encourage plant growth, which will decrease with increasing plant age. This statement is not in accordance with this study, as the auxin content increased again at week 8th. This is presumably due to the use of mother plants as material for analyzing auxin content from different parent plants every week. These results cause differences in growth and auxin content in shoots that are not similar. Auxin content is affected by changes in growth. If the endogenous hormone has a low value, it is necessary to give exogenous hormones to get a maximum response to the explants. Administration of exogenous hormones is needed to meet the hormone needs of explants during the root induction process (Supriyanto and Prakasa 2011). The addition of exogenous auxin will also increase endogenous auxin activity in plants, thus encouraging cell division and causing earlier shoot growth (Tamba et al. 2019).

Percentage survival of explants

The survival percentage of rubber plant shoot explants from the 2nd to the 4th week of planting was low, indicating that the addition of IBA and NAA did not have a significant effect on the survival percentage (Table 2) (P>0.05; Appendix). The low survival rate during this period was attributed to the loss and decay of explants, leading to their death.

Symptoms of leaf loss begin with a change in leaf color. Initially, the leaves are green, but they gradually turn yellow, wither,

Table 2. Percentage of survival rate of explants for 16 weeks.

Treatment (ppm)	Week of Observation							
	2	4	6	8	10	12	14	16
IBA 0 + NAA 0	50	16.6	16.6	16.6	16.6	1 6.6	16.6	16.6
IBA 0 + NAA 50	58.3	25	0	0	0	0	0	0
IBA 0 + NAA 100	83.3	16.6	0	0	0	0	0	0
IBA 0 + NAA 150	91.6	0	0	0	0	0	0	0
IBA 100 + NAA 0	75	0	0	0	0	0	0	0
IBA 100 + NAA 50	66.6	8.3	8.3	8.3	8.3	8.3	8.3	8.3
IBA 100 + NAA 100	83.3	8.3	8.3	8.3	0	0	0	0
IBA 100 + NAA 150	83.3	33.3	8.3	0	0	0	0	0
IBA 200 + NAA 0	91.6	16.6	16.6	16.6	8.3	8.3	8.3	8.3
IBA 200 + NAA 50	75	16.6	16.6	8.3	8.3	8.3	8.3	8.3
IBA 200 + NAA 100	83.3	16.6	0	0	0	0	0	0
IBA 200 + NAA 150	58.3	0	0	0	0	0	0	0
IBA 300 + NAA 0	41.6	16.6	16.6	8.3	8.3	8.3	8.3	8.3
IBA 300 + NAA 50	75	16.6	16.6	16.6	16.6	16.6	16.6	16.6
IBA 300 + NAA 100	25	0	0	0	0	0	0	0
IBA 300 + NAA 150	50	16.6	16.6	16.6	16.6	16.6	16.6	16.6
IBA 400 + NAA 0	50	8.3	8.3	8.3	8.3	8.3	8.3	8.3
IBA 400 + NAA 50	83.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
IBA 400 + NAA 100	58.3	8.3	0	0	0	0	0	0
IBA 400 + NAA 150	100	25	0	0	0	0	0	0
IBA 500 + NAA 0	100	16.6	0	0	0	0	0	0
IBA 500 + NAA 50	100	25	0	0	0	0	0	0
IBA 500 + NAA 100	100	0	0	0	0	0	0	0
IBA 500 + NAA 150	100	0	0	0	0	0	0	0

and eventually fall off. Meanwhile, root symptoms at the base of the stem are characterized by a color change from green to black, spreading upwards, and causing the stem to dry and turn brown. The loss and decay observed during the second to fourth week are believed to be a result of the explants still undergoing an adaptation process. Explants require time to adjust to changes in environmental conditions. During this adaptation process, wound healing occurs at the base of the stem. In such conditions, shoot explants are more prone to death (Setyayudi 2018). The survival of shoot explants until the end of the observation period can be attributed to the sufficient availability of carbohydrates within the explants (Pujawati et al. 2017). Carbohydrates play a crucial role in plant growth according to previous research (Pujawati et al. 2017).

The percentage of explant survival is influenced by both internal and external factors. Internal factors encompass the carbohydrate content in the explant tissue, water availability, explant age, endogenous hormones, and plant species. On the other hand, external factors include the choice of media, humidity, temperature, light intensity, and planting techniques (Danu et al. 2018). Explants rely on carbohydrates for their survival, which are primarily produced through photosynthesis in leaves. However, leaf loss in this study hindered the photosynthesis process, resulting in a lack of available carbohydrates within the explants, ultimately leading to their death (Supriyanto and Prakasa 2011). The low survival percentage is thought to be attributed to inadequate water availability, which affects nutrient absorption from the soil, resulting in a lack of carbohydrates within the explants (Rianto et al. 2016). The role of water in explants lies in nutrient transportation from the soil to the plants. Increased nutrient absorption supports the availability of essential components for the photosynthesis process, such as carbohydrates (Rianto et al. 2016). Consequently, the loose leaves on the explants impede the photosynthesis process and contribute to the unavailability of carbohydrates.

In this study, the explants used were 6 weeks old, and their age had an impact on the survival percentage. Younger explants displayed better root formation ability compared to older ones due to their higher cell elongation capacity (Darwo et al. 2018). The percentage of survival in rubber plant explants was lower due to the disruption of hormone supply during pruning, impeding the regeneration process required for normal plant development (Supriyanto and Prakasa 2011). Rubber trees contain sap that hinders the distribution of carbohydrates and hormones, affecting their availability. The presence of undrained sap at the base of the stem leads to high mortality rates by hindering root growth (Saijo 2015).

External factors that impact the survival rate of explants include the media, humidity, temperature, light intensity, and explant planting techniques (Danu et al. 2018). The findings revealed that the morning temperature ranged from 26.5-31.9 ºC, daytime temperature ranged from 29.6-33.9 ºC, and afternoon temperature ranged from 26.6-33.5ºC. Humidity observations indicated that morning humidity ranged from 74.7-86.5%, daytime humidity ranged from 63-86.5%, and afternoon humidity ranged from 62.1-85.5% (Appendix 3). These results indicate that the environmental conditions in the containment are not always optimal, and suboptimal conditions can lead to the death of explants (Pujawati et al. 2017). Rubber plants require temperatures between 26-32 ºC and a humid environment (Harahap and Segoro 2018). The use of a 65% shading net is considered inadequate in blocking the amount of light entering the incubator, leading to high temperatures and low humidity. Consequently, explants experience excessive transpiration, resulting in dryness and death (Sukendro and Putri 2016).

According to Danu et al. (2011), the density of the media hinders the ability of plant roots to absorb water and reduces soil porosity. This condition affects water and air translocation in the metabolism of explants, which is crucial for their survival. Additionally, the planting technique employed in this study is deemed inaccurate, particularly in relation to the pruning point. In this study, pruning was conducted approximately 30 cm above the ground. Improper pruning results in shoots that are not yet juvenile. Research by Putri (2009) indicates that pruning points as high as 30 cm on dahu plants have a low percentage of life, which is related to the level of plant juvenility caused by pruning techniques.

Number of Leaves

The number of leaves in explants decreased between the 2nd and 4th weeks after planting. However, at the 16th week, explants treated with IBA 200 ppm + NAA 50 ppm and IBA 400 ppm + NAA 0 ppm exhibited an increase in the number of leaves (Table 3), evident from the growth of new shoots. This can be attributed to the formation of calli or roots at the stem's base. Formed roots in explants facilitate greater carbohydrate absorption, which supports the growth of new shoots and leaves (Nuroniah et al. 2018). The formation of shoots in explants is influenced by carbohydrate and auxin content, where higher levels of carbohydrates and auxin promote shoot production (Pujawati et al. 2017).

The low number of leaves in explants can be attributed to leaf loss, explant death, and the absence of shoot formation. As a result, carbohydrates are depleted in explants, while transpiration continues through the leaves. This leads to a lack of

Table 3. Average number of leaves on rubber explants for 16 weeks

Treatment (ppm)	Week of Observation							
	2	4	6	8	10	12	14	16
IBA 0 + NAA 0	0.75	0.58	0.58	0.58	0.58	0.58	0.58	0.58
IBA 0 + NAA 50	1.25	0	0	0	0	0	0	0
IBA 0 + NAA 100	2.67	0	0	0	0	0	0	0
IBA 0 + NAA 150	1	0	0	0	0	0	0	0
IBA 100 + NAA 0	0.33	0	0	0	0	0	0	0
IBA 100 + NAA 50	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08
IBA 100 + NAA 100	1.91	0.33	0.33	0.16	0	0	0	0
IBA 100 + NAA 150	2.41	0.33	0.08	0	0	0	0	0
IBA 200 + NAA 0	2.41	0.16	0.16	0.16	0.08	0.08	0.08	0.08
IBA 200 + NAA 50	1.16	0.58	0.25	0.25	0.25	0.25	0.25	0.5
IBA 200 + NAA 100	1.75	0	0	0	0	0	0	0
IBA 200 + NAA 150	0.75	0	0	0	0	0	0	0
IBA 300 + NAA 0	0.83	0.67	0.25	0.16	0.16	0.16	0.16	0.16
IBA 300 + NAA 50	1.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
IBA 300 + NAA 100	0.58	0	0	0	0	0	0	0
IBA 300 + NAA 150	1.83	1.16	0.91	0.91	0.91	0.91	0.91	0.91
IBA 400 + NAA 0	1	0.16	0.08	0.08	0.08	0.08	0.08	0.41
IBA 400 + NAA 50	0.41	0.33	0.33	0.33	0.33	0.25	0.16	0.16
IBA 400 + NAA 100	0.91	0	0	0	0	0	0	0
IBA 400 + NAA 150	0.41	0	0	0	0	0	0	0
IBA 500 + NAA 0	0.33	0	0	0	0	0	0	0
IBA 500 + NAA 50	1.08	0	0	0	0	0	0	0
IBA 500 + NAA 100	0	0	0	0	0	0	0	0
IBA 500 + NAA 150	0.25	0	0	0	0	0	0	0

water intake, nutrients, and minerals, causing the leaves to wither and fall off, ultimately resulting in the death of the explants. The absence of shoot formation is caused by inadequate root growth, leading to a lack of food and water for the explants (Pujawati et al. 2017). Inadequate root growth causes the explants' food reserves to rely solely on carbohydrates in the stem, stunting shoot growth (Saijo 2015).

Root formation

The formation of rubber plant roots after induction resulted in a low percentage, number, and length of roots. The addition of IBA and NAA to induce root formation in rubber shoot explants did not show a significant effect (P>0.05; Table 4).

The limited ability of explants to induce root formation was attributed to insufficient carbohydrates and endogenous hormones. A deficiency of carbohydrates and hormones in the explants hindered root growth. Explants require an adequate supply of carbohydrates for cell division and root development (Pujawati et al. 2017). Explants that eventually formed roots at the end of the observation were those treated with IBA 200 ppm + NAA 0 ppm, IBA 200 ppm + NAA 50 ppm, IBA 300 ppm + NAA 50 ppm, and IBA 400 ppm + 0 ppm. Some treatments demonstrated that explants could survive even without inducing root formation. This was because the carbohydrate content in the explants was sufficient for survival but insufficient to trigger root formation (Supriyanto and Prakasa 2011).

Explants that survived but failed to develop roots were those treated with IBA 0 ppm + NAA 0 ppm and IBA 100 ppm + NAA 50 ppm. Callus formation occurred at the base of the stem in some explants. Callus formation was observed in explants treated with IBA 300 ppm + NAA 150 ppm and IBA 400 ppm + NAA 50 ppm (Figure 6). The presence of callus on the explants indicated the potential for root formation, but the addition of IBA and NAA was not optimal, leading to poorly differentiated callus on the stem of the explants (Pujawati et al. 2017).

In contrast, a different study by Ulya et al. (2019) utilized explants derived from young plants obtained from seeds. They

Table 4. Results of root formation in rubber explants

Treatment	Root Percentage (%)	Number of Root	Length of Root (cm)
IBA 0 + NAA 0	0	0	0
IBA 0 + NAA 50	0	0	0
IBA 0 + NAA 100	0	0	0
IBA 0 + NAA 150	0	0	0
IBA 100 + NAA 0	0	0	0
IBA 100 + NAA 50	0	0	0
IBA 100 + NAA 100	0	0	0
IBA 100 + NAA 150	0	0	0
IBA 200 + NAA 0	8.30	1.00	2.80
IBA 200 + NAA 50	8.30	3.00	10.70
IBA 200 + NAA 100	0	0	0
IBA 200 + NAA 150	0	0	0
IBA 300 + NAA 0	0	0	0
IBA 300 + NAA 50	16.60	3.00	4.20
IBA 300 + NAA 100	0	0	0
IBA 300 + NAA 150	0	0	0
IBA 400 + NAA 0	8.30	1.00	3.90
IBA 400 + NAA 50	0	0	0
IBA 400 + NAA 100	0	0	0
IBA 400 + NAA 150	0	0	0
IBA 500 + NAA 0	0	0	0
IBA 500 + NAA 50	0	0	0
IBA 500 + NAA 100	0	0	0
IBA 500 + NAA 150	0	0	0

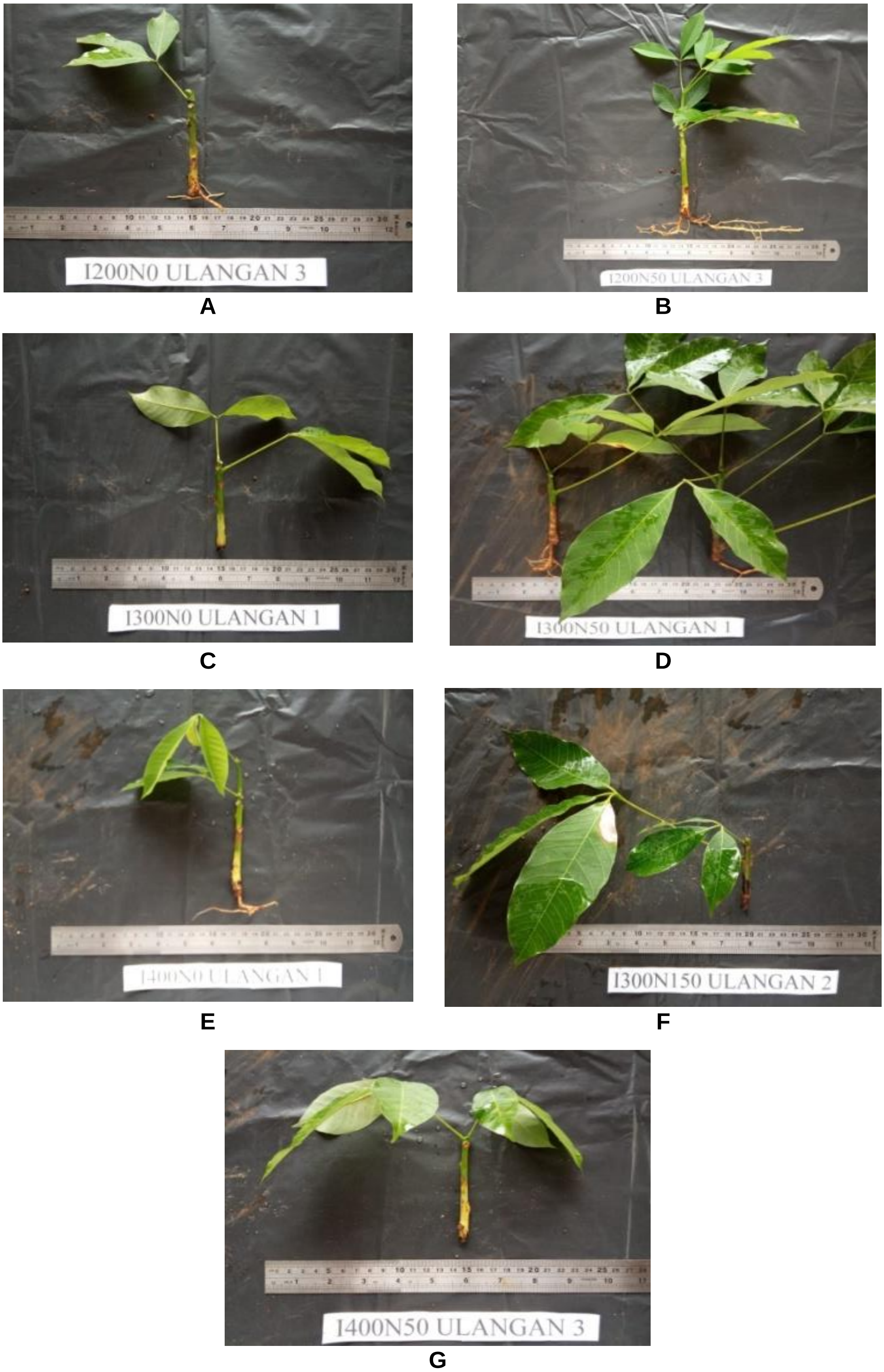

Figure 4. Rubber explants showing roots producing callus. Root formation in treatment of: (A) IBA 200 ppm + NAA 0 ppm, (B) IBA 200 ppm + NAA 50 ppm, and (D) IBA 300 ppm + NAA 50 ppm, and (F) IBA 400 ppm + NAA 0 ppm; callus formation in treatment with: (C) 300 ppm IBA + 0 ppm NAA, (E) 300 ppm IBA + 150 ppm NAA, and (G) 400 ppm IBA + 50 ppm NAA

found that using brown stems with an IBA concentration of 100 ppm resulted in the highest rooted percentage of 54.84% and a root length of 7.93 cm. On the other hand, green stems showed the best results with a treatment of 300 ppm IBA, yielding a rooted percentage of 34.38%.

CONCLUSION

The study was of IBA and NAA and their interaction on *ex vitro* PB 260 clone rubber explants could not significantly affect the induction of root formation. The highest root induction was obtained in the combination of IBA 300 ppm and NAA 50 ppm treatment of 16.60%. It is necessary to optimize other auxin concentrations to get optimal rooted success.

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