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Research Article

Analysis the effect of plant growth regulator (Gibgro-20T) on seaweed *Eucheuma cottonii* by in vitro

Analisis pengaruh zat pengatur tumbuh (Gibgro-20T) terhadap rumput laut *Eucheuma cottonii* secara in vitro

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ABSTRACT. This study aims to determine the effect of the dose of plant growth regulator (PGR) Gibgro-20T on the growth of *Eucheuma cottonii* in-vitro. The experimental method used was a Completely Randomized Design (CRD) treatment with a Gibgro-20T dose of 0 ppm (control), 5 ppm, 10 ppm, and 15 ppm. The results showed that the highest weight growth rate (RGR_W) obtained at 10 ppm PGR dose, which is 1.27% per day. Meanwhile, the highest length growth rate (RGR_L) obtained at a dose of 10 ppm, which was 1.72% per day, and the highest RGR_L was significantly different from the control (0 ppm) and 5 ppm but was not different real ($P<0.05$) with 15 ppm treatment. Using Gibgro-20T at doses of 5, 10, and 15 ppm, however, doses of 10 and 15 ppm show higher length growth.

ABSTRAK. Penelitian ini bertujuan untuk mengetahui pengaruh dosis Zat Pengatur Tumbuh (ZPT) Gibgro-20T terhadap pertumbuhan *Eucheuma cottonii* secara in-vitro. Metode eksperimen yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan dosis Gibgro-20T 0 ppm (kontrol), 5 ppm, 10 ppm, dan 15 ppm. Hasil penelitian menunjukkan bahwa laju pertumbuhan bobot (RGR_W) tertinggi diperoleh pada dosis ZPT 10 ppm yaitu 1,27% per hari. Sedangkan laju pertumbuhan panjang (RGR_L) tertinggi diperoleh pada dosis 10 ppm yaitu 1,72% per hari, dan RGR_L tertinggi berbeda nyata dengan kontrol (0 ppm) dan 5 ppm tetapi tidak berbeda nyata ($P<0,05$) dengan perlakuan 15 ppm. Penggunaan Gibgro-20T pada dosis 5, 10, dan 15 ppm, namun pada dosis 10 dan 15 ppm menunjukkan pertumbuhan panjang yang lebih tinggi.

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1. Introduction

One of the obstacles in seaweed cultivation is environmental factors that can change every season. In the dry season, marine algae look dwarfed and break easily, while in the rainy season, it is susceptible to ice-ice disease and a soft thallus texture (Suryanti *et al.*, 2007). According to Mulyaningrum *et al.* (2012), another obstacle faced in developing marine algae cultivation is the limited quality of continuous seeds.

Many cultivators get new seeds, namely by taking cuttings from existing marine algae. Continuous use of seeds without a selection will cause a decline in seed quality. Plant micro propagation development through organ culture techniques, tissue culture, and cell culture provide promising prospects for developing plant biotechnology and great opportunities for genetic manipulation, plant propagation, and commercial crop production. In vitro marine algae tissue culture using callus induction techniques is widely used for clone propagation and genetic quality improvement to support continuous and quality seed availability.

Callus induction is one of the stages involved in somatic embryogenesis. Callus induction is very important in tissue culture activities because it is through this stage that determines the next stage, for example, somatic embryogenesis, genetic improvement, cell culture, secondary metabolite production, and to obtain intact plants (Santoso & Nursandi, 2003). The nutrients contained in the maintenance media are one of the factors that influence the growth of seaweed. Insufficient nutrients can be supplied using fertilizers. Fertilization can be done indirectly, namely by soaking seaweed in a fertilizer solution before the maintenance process. Sufficient nutrients are used by seaweed to grow through the photosynthesis process. Fertilizer application can be done by soaking seaweed prior to maintenance (Lideman *et al.*, 2013), however, fertilizer application must be at the appropriate dose for seaweed growth.

Gibgro-20T is a plant growth regulator (PGR) used for seed treatment. This product contains the active ingredient gibberellic acid 20%, which functions to accelerate shoot growth and use in the vegetative and generative phases to help plant development (Isbiyantoro *et al.*, 2015). This study uses plant growth regulator (PGR) due to its use which is more environmentally friendly, affordable to the community and contains the nutrients that seaweed needs. Based on these descriptions, this study conducted to determine the effect of Gibgro-20T plant growth regulator on the growth rate of *E. cottonii* in vitro.

2. Materials and Method

2.1. Research Sites

This research was conducted at the Seaweed Network Culture Laboratory of the Takalar Brackish Water Cultivation Center, Ministry of Marine Affairs and Fisheries, Mappakalompo Village, Galesong Selatan District, Takalar Regency, Indonesia.

2.2. Preparation of Tools and Materials

The tools used in this study were Erlenmeyer flask, tissue, spray bottle, slice board, autoclave, thermometer, analytical scale, cutter, tweezers, sponge, basket, table cloth, oven, magnetic stirrer and hot plate, scoop, tea filter, refrigerator, Erlenmeyer Cleaning Brush, filter paper, scale pipette, vacuum pump, ruler, measuring cup, alcohol lamp, mask, camera, cellphone. While the materials used in this study were *E. cottonii* seaweed, PGR Gibgro-20T, sterile seawater, betadine, sterile freshwater, aluminum foil, and labels.

2.3. Experimental Design

This study was an experimental study with a completely randomized design (CRD) consisting of four treatments and three repetitions (Table 1). The treatments are as follows:

Treatment A = Gibgro-20T 0 ppm
Treatment B = Gibgro-20T 5 ppm
Treatment C = Gibgro-20T 10 ppm
Treatment D = Gibgro-20T 15 ppm

Table 1. Treatment Design

Treatment	Deuteronomy		
	1	2	3
A	A_1	A_2	A_3
B	B_1	B_2	B_3
C	C_1	C_2	C_3
D	D_1	D_2	D_3

Each treatment repeated three times so that there were 12 experimental units in total. The CRD experimental units are limited by the rooms, so that there will be no interaction between each research unit. Therefore, each research unit's location will not affect the results of the experiment because this experiment was carried out under controlled conditions. The method used in placing the layout of the research container is to use random numbers. The layout of the research container is determined based on a table of numbers. Random can be seen in Figure 1.

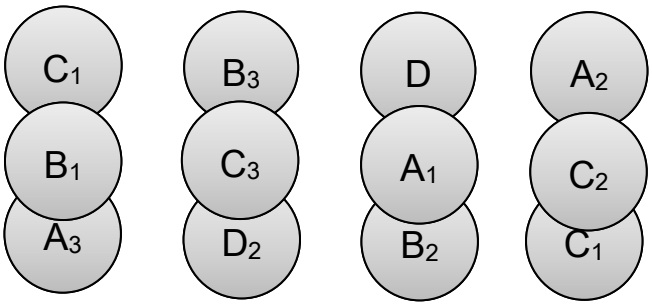

Figure 1. Layout of the Research Container.

2.4. Analytical Procedure

Measurement of explant weight was conducted by weighing explants once a week for 42 days of maintenance using a 4-digit electric scale. The Relative Growth Rate (RGR) was calculated using the formula (1) (Effendie, 1978).

$$RGR\ (\%\ per\ day) = [(\ln L1 - \ln Lo) / (t)] \times 100 \quad (1)$$

Notes: RGR = Relative Growth Rate of Length (L) or Weight (W), Lo = Initial Length or Weight, L1 = the final Length or Weight, t = Time.

The Survival Rate (SR) of seaweed was calculated using the formula (2).

$$SR\ (\%) = (Nt/No) \times 100 \quad (2)$$

Notes: SR = Survival Rate, Nt = Number of live explants on day t, No = Number of explants at the start of maintenance.

2.5. Data Analysis

The data obtained in this study are displayed in tables and graphs. The parameters of RGR_W, RGR_L and SR are calculated based on the results of the sampling weight, length and survival rate of the *E. cottonii* explants. To determine each dose treatment's effect on the parameters of RGR_W, RGR_L, analysis of variance (ANOVA) was used. If ANOVA shows a significant difference ($P<0.05$), it is done with the LSD advanced test. To find out the differences between treatments.

3. Results and Discussion

3.1. Explant Weight Growth

Data obtained from weighing and measuring explants seven times during (42 days), obtained from weighing and measuring explants every week and tested using the variance test or ANOVA to determine the ratio of different doses using the Gibgro-20T plant growth regulator to the parameter of developmental observations. Explant weight. The results of observing the development of explant weight can be seen in Figure 2.

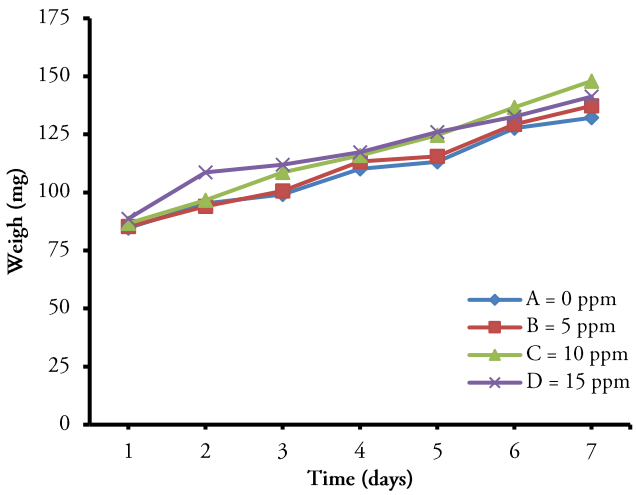

Figure 2. Average Weight Development of *Eucheuma cottonii* explants during 42 days of maintenance.

The results in Figure 2 show that the average value of explant weight development at the beginning of the study is 84-88 mg from each treatment, which has increased relatively stable every week. The highest average length development at the 10 ppm dose of treatment experienced a relatively stable increase compared to other treatments. Followed by treatment of 15 ppm and experienced the highest increase in the second week. In the 5 ppm treatment, the average weight development increased, which was relatively stable. In the control treatment 0 ppm, the weight increased less than the other treatments and decreased at week 4.

The results in Figure 3 show the weight growth rate of *E. cottonii* seaweed explants with different doses each week. Without control, or 0 ppm treatment experienced a relative weight growth rate of 1.12% per day. 5 ppm treatment with a weight growth rate of 1.13% per day. While the 10 ppm treatment obtained a weight growth rate of 1.27% per day, and finally, in the 15 ppm treatment, the weight growth rate was 1.21% per day. The highest relative weight growth rate at 10 ppm treatment is 1.27% per day, followed by the treatment of 15 ppm and 5 ppm with growth rates of 1.21 and 1.13% per day, respectively, while the lowest growth rate, and was obtained in the 0 ppm treatment or control, namely 1.12% per day. The statistical analysis of the variance test showed the

difference in dosage in the treatment of plant growth regulators (PGR). That did not significantly affect the weight growth rate, which was because the significant value in the ANOVA table was 0.330, which means it was more significant than the α0.05 level.

The initial length of the thallus *E. cottonii* used in the study was 2 cm. The length of development at 10 ppm dose of treatment increased relatively high compared to other treatments, and the peak increased in the last week. Followed by the treatment, the dose of 15 ppm has increased, which is relatively stable and has increased in the last week. For the dose of 5 ppm, the dosage is almost the same every week. The lowest average length development was without treatment (control), namely 0 ppm, but increased at week 5. The growth rate of explants length per day during maintenance can be seen in Figure 5.

The growth rate data for explant length in Figure 5 shows that the length of the explants produced during the study was 42 days with the administration of PGR Gibgro-20T doses in different treatments. At 0 ppm treatment or control, the length growth rate was 0.94% per day. At 5 ppm treatment, the length growth rate was 1.05% per day. Furthermore, for treatment 10 ppm, the growth rate is 1.75% per day, and finally, for treatment 15 ppm, the growth rate is 1.40% per day. The treatment that showed the highest length growth rate was the 10ppm treatment of 1.75% per day. Then followed by the treatment of 15 ppm and 5 ppm with growth rates of 1.40 and 1.05% per day. Simultaneously, the treatment with the percentage level for the lowest growth rate of explant length was obtained at 0 ppm treatment or control as much as 0.94% per day. The statistical analysis of the variance test showed that the different treatment doses in the growth regulator solution had a significant effect on the explant length's growth rate. The significant value in the ANOVA table was 0.027, which means it is smaller than the α0.05 level. Then proceed with the test. Smallest Real Difference (LSD). The highest treatment for length growth rate obtained at 10 ppm treatment was significantly different from treatment 5 and 0 ppm (control) but not significantly different from treatment 15 ppm; for treatment, 15 ppm was significantly different from 5 and 0 ppm or control but not different. Significantly with 10 ppm, while for treatment, 5 ppm was not significantly different from 0 ppm (control).

The explant growth influenced by several factors, namely the surrounding environment and its nutrients. According to Susanto *et al.* (1996) one of the efforts that can be done to stimulate the growth of seaweed is the addition of plant growth regulator (PGR). The research results giving Gibgro-20T plant growth regulator (PGR) in the growth of seaweed in vitro with PGR administration did not show significant differences in each treatment. Each treatment did not show a significant increase in each treatment, and it was shown in the 0 ppm treatment, the growth rate was 1.12% per day. They were then followed by the 5 ppm treatment, where the growth rate was 1.13% per day, and 10 ppm treatment was 1.18% per day. Whereas in 0 ppm treatment or control, as much as 0.12% per day. According to Yuliana *et al.* (2013) seaweed is different from land plants, seaweed does not have roots to absorb nutrients, so the availability of nutrients around the thallus will significantly affect growth. Lack of nutrients will usually cause seaweed to become stunted. Seaweed requires nutrients for growth and survival. The low growth is thought to be due to insufficient nutrient availability for *C. lentillifera* so that the addition of fertilizers will provide good growth results (Ginting *et al.*, 2015). According to Yuliana *et al.* (2013), cell regeneration in each explant to form a complete thallus can only occur if the explants live in media that contains sufficient nutrients. Seaweed requires nutria. Thus, according to (Hayashi *et al.*, 2008), Plant Growth regulators (PGR) are non-nutrient organic compounds, which in small amounts can support, inhibit, and change plant physiological processes. Plant growth regulator (PGR) Gibgro-20T is thought not to be too optimal in weight gain of seaweed explants because these growth regulators' function is only to stimulate the growth of morphogenesis in cell, tissue and organ cultures but not too much influence in weight gain.

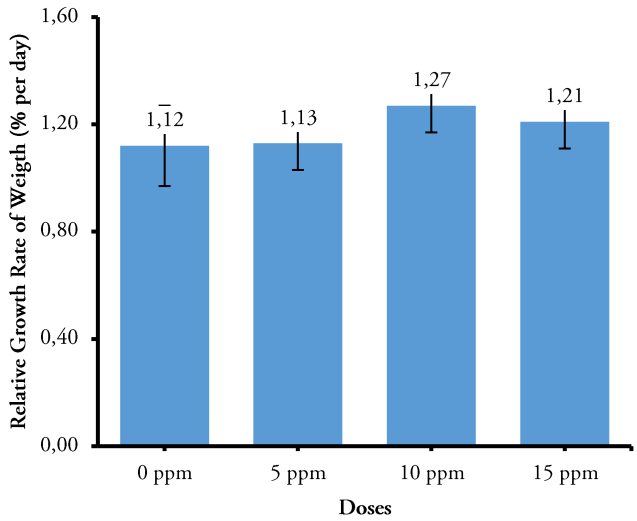

Figure 3. Relative Weight Growth Rate (RGRW) of *Eucheuma cottonii* explants 42 days of maintenance.

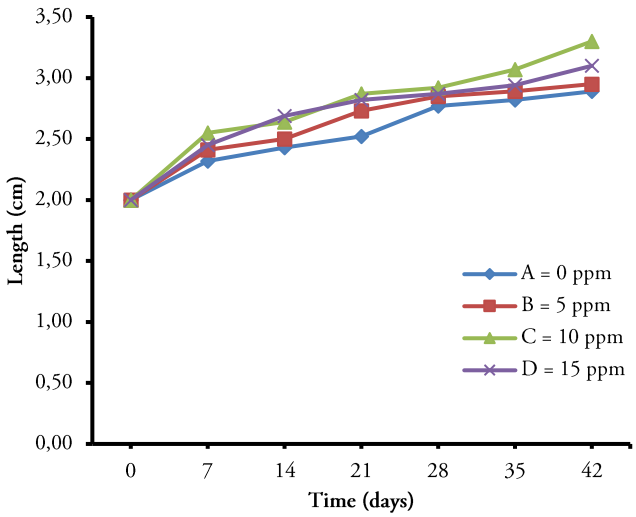

Figure 4. Average development of *Eucheuma cottonii* explant length during 42 days of maintenance.

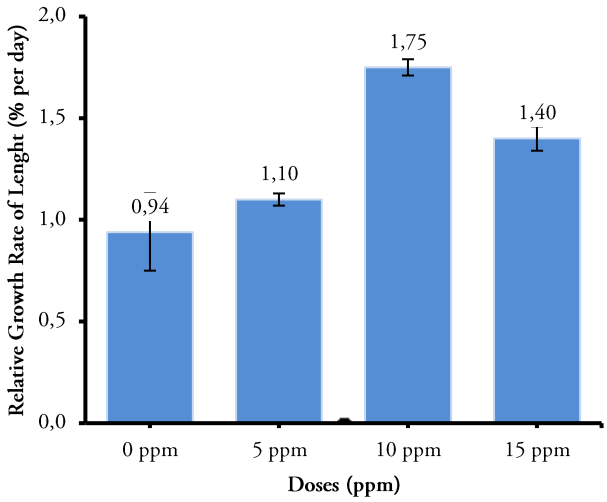

Figure 5. Explant-long Relative Growth Rate (RGRL) in 42 days of maintenance.

3.2. Survival Rate (SR)

The synthase or survival rate of seaweed explants for 42 days treated with a plant growth regulator (PGR) Gibgro-20T at the time of maintenance showed the same results, namely a very high survival rate of 100% (no death), in contrast to controls who had a synthase of 86.3% (Figure 6). Because in the control treatment of 15 explants in three plates, two explants died, so there was a decrease in the control diet's survival rate. So that in this study, it was proved that the administration of plant growth regulator gave a better effect than control. Thus is suspected because the control not given a plant growth regulator (PGR), so that the absorption of nutrients is deficient in long or heavy growth in seaweed.

Gibgro-20T contains 20% gibberellic acid. Gibberellic acid is a class of phytohormones found in not only in inland animals or plants but also in marine organisms. This phytohormones has reported being one of the exogenous PGRs. The biological and physiological functions of PGR on growth, development, and environmental stimuli have been reported previously (Wei *et al.*, 2020). Shi *et al.* (2015) reported that the application of exogenous PGR melatonin could improve salt resistance, drought, and cold stress in Bermuda grass [*Cynodon dactylon* (L). Pers.] by relieving respiratory burst and cell damage due to reactive oxygen species.

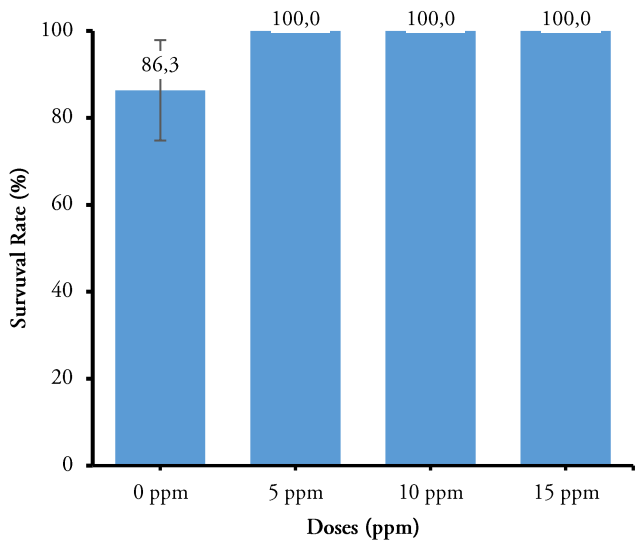

Figure 6. Survival Rate (SR) of Seaweed Explants.

3.3. Water Quality

Water quality is an essential factor in the growth of explants grown in culture media. Measurement of water quality in this study carried out when changing the culture media. Water quality parameters during the study can be seen in Table 5.

Table 5. Water Quality Parameters of Culture Media.

Parameters	Value
Temperature	24–26 °C
Light Intensity	300 lux
Salinity	30 ppt

Factors that affect the survival of the explants during maintenance include temperature, salinity, light availability, and the culture room's sterility. During the study, the results of temperature measurements were 24-26 °C. Yuliana *et al.* (2013) supported this statement, which states that temperature is an essential factor for growth and reproduction, the optimum temperature for growth *E. cottonii* between 20-28ºC. Several studies have shown that *Eucheuma serra* requires a temperature of 24-28 ºC for its in vitro growth (Lideman *et al.*, 2013), while *Kappaphycus* sp. (Sumba strain) requires a temperature of 22-23 ºC and sunlight intensity between 122-167 µmol photons m^{-2} s^{-1} (Lideman *et al.*, 2013). During the maintenance period, salinity measurements, namely 30 ppt, were used in the explant maintenance media. This salinity follows the statement stated by Arisandi *et al.* (2013) that *E. cottonii* is a seaweed that lives well with waters with a salinity of 30-37 ppt. According to Choi *et al.* (2010) that water quality parameters play a role in the growth, thallus formation, and morphogenetic development of seaweed algae is salinity because it is directly related to the osmoregulation that occurs in cells. The different density between the fluid inside and outside the cell encourages the Golgi apparatus to keep trying to balance until it becomes isotonic. Meanwhile, the light intensity obtained during this study was around ±300 lux. *E. cottonii* was still able to live in that range of light intensity.

4. Conclusion

The application of Gibgro-20T growth regulators to the maintenance medium did not significantly affect the weight growth rate for *E. cottonii.* The highest weight growth rate (RGR_W) obtained at a dose of PGR 10 ppm, which was 1.27% per day; however, the highest RGR_W was not significantly different (P<0.05) with control (0 ppm), 5 and 15 ppm. Giving Gibgro-20T growth regulators (PGR) had a significant effect on the length growth rate of the *E. cottonii* thallus. The highest length growth rate (RGR_L) obtained at a dose of 10 ppm, which was 1.72% per day, and the highest RGR_L was significantly different from the control (0 ppm) and 5 ppm but was not significantly different (P<0.05) with treatment 15 ppm.

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Competing interest
All author(s) declare no competing interest.

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