Plant Spacing Variations on Cell Number and Agar Content in *Gracilaria verrucosa*

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Abstract

The primary issue at the Gracilaria verrucosa seaweed cultivator is the low productivity associated with current cultivation methods. Analyzing cell number and agar content provides valuable insights into seaweed cultivation productivity, growth activity, nutrient absorption, and chemical composition. This research aims to examine how variations in planting distance affect the cell number and agar content of Gracilaria verrucosa. The research was conducted at the Maranak Experimental Pond Installation, Brackish Water Aquaculture Fisheries Research Institute, located in Marannu Village, Lau District, Maros Regency. Analysis of cell numbers and agar content was performed at the Fisheries Research Institute Laboratory of the Brackish Water Aquaculture Center from June to July 2024. Utilizing a completely randomized quantitative experimental design with four treatments and three repetitions, the research included: Treatment A (planting distance 20 cm), Treatment B (30 cm), Treatment C (40 cm), and Treatment D (50 cm). The results indicated that planting distance significantly impacts cell number, with the 50 cm distance yielding the highest average cell count of 213.00 ± 9.64. Similarly, planting distance affects agar content, with the 50 cm distance producing the highest agar content of 27.31%. Therefore, a planting distance of 50 cm is identified as the most effective, resulting in both the highest number of cells and agar content.

Keywords*: Gracilaria verrucosa;* Number of Cells; Agar Content*; Seaweed cultivation methods.*

INTRODUCTION

Seaweed is a valuable aquaculture commodity with significant contribution to total aquaculture production in South Sulawesi Province, with production reaching 1,009,275.3 tons in 2020 (BPS, 2021). South Sulawesi ranks as the second-largest seaweed producer in the world, following Chile (Pessarrodona *et al*., 2022). Seaweed holds economic value as a source of foreign exchange and as a labor-intensive industry, providing substantial employment opportunities (Farghali *et al*., 2023; Yun *et al*., 2023). Its unique chemical properties and rapid growth rate suggest the potential for seaweed-based bio-products to replace fossil-based products, offering financial viability for commercialization (Nakhate & van der Meer, 2021). *Gracillaria verrucosa* is a particularly promising type of seaweed, containing agar used in the pharmaceutical, industrial, and food sectors (Mendes *et al*., 2022).

Unfortunately, *Gracillaria verrucosa* cultivation is hampered by several challenges, including low productivity due to seed quality, cultivation methods, and environmental factors (Jiksing *et al*., 2022) (Sun *et al*., 2023) . The growth and productivity of *Gracillaria verrucosa* are heavily influenced by various environmental factors, with planting distance playing a crucial role in determining population density and interactions among individual thalli (Annas *et al*., 2019; Asaad *et al*., 2024). Current cultivation methods yield low productivity levels, and there is limited physiological research on cell count and quality changes in *Gracillaria verrucosa* related to planting distance. Effective cultivation methods are needed to optimize *Gracillaria verrucosa* seaweed production.

In general, the cultivation of Gracillaria verrucose seaweed in South Sulawesi is still carried out in a traditional way, is simple, and has not received much technological input from outside (Agustang et al., 2019). Saputro *et al.* (2021) examined the effect of planting distance on the specific growth rate of *Gracillaria verrucosa*, while (Fanni., 2021; Sugandi *et al*., 2019)studied its impact on seaweed production. (Annas *et al*., 2019) investigated how different planting distances affect cell count and quality. Previous research focused on growth parameters, production, cell count, and agar content, but these studies were conducted outside South Sulawesi. No research has yet physiologically examined changes in *Gracillaria verrucosa* cell count with varying planting distances in South

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Sulawesi. This research aims to analyze changes in cell number and agar content of *Gracillaria verrucosa* in response to different planting distances.

MATERIALS DAN METHODS

This quantitative experimental research was performed using a Completely Randomized Design (CRD) with four treatments and three replications with a total of 12 experimental units. The plant spacing treatments used in the research are detailed in *Table* 1.

The research was conducted at the Maranak Experimental Pond Installation, Brackish Water Aquaculture Fisheries Research Institute, located in Marannu Village, Lau District, Maros Regency. The analysis of cell counts and content was performed at the Fisheries Research Institute Laboratory of the Maros Brackish Water Cultivation Center. The test material consisted of *Gracillaria verrucosa* seaweed seeds, which were sourced from the Maranak Experimental Pond Installation. The cultivation was carried out in a pond with an area of 0.2 hectares (2000 m²).

The seeds used were young, brightly colored thalli from the tips of the seaweed. They were washed thoroughly to remove any attached dirt and then weighed, with an initial weight of 50 grams (Fanni *et al*., 2021). The longline cultivation method was employed, with planting conducted in the morning (Yusuf *et al*., 2021). The seaweed seeds were secured with raffia rope, with spacing between plants determined by the experimental treatments. The distance from the seaweed to the water's surface was maintained at 30 cm. The *Gracillaria verrucosa* seaweed was cultivated for 49 days, during which control and cleaning procedures were carried out. The planting model for *Gracillaria verrucosa* seaweed is illustrated in Figure 1.

Table 1. Different Distances in the Treatments

Treatment	Planting Distance
A	20 cm
B	30 cm
C	40 cm
D	50 cm

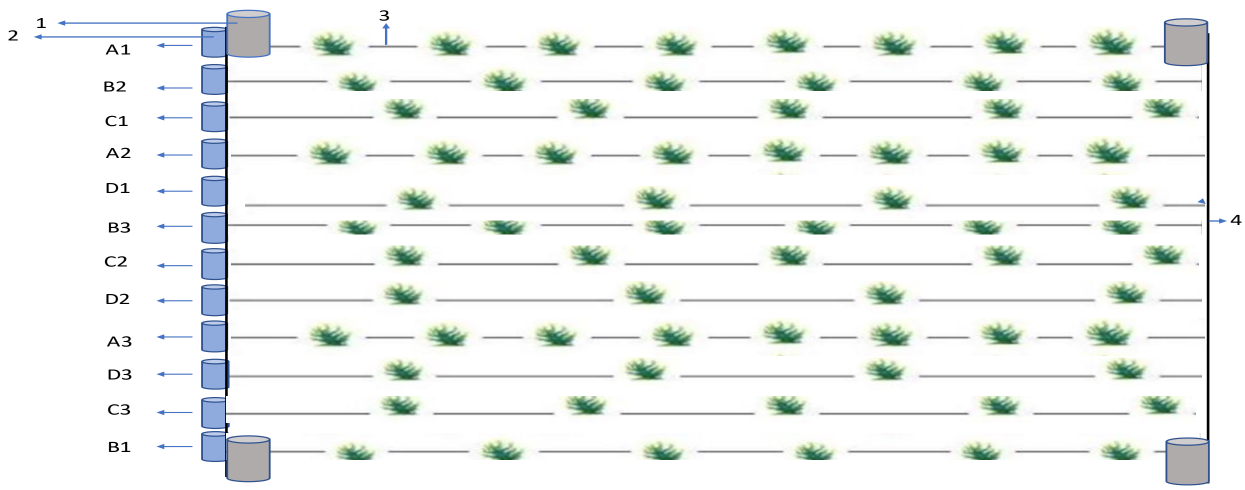

Figure 1. Seaweed Planting Model
Note: 1 = Bamboo, 2 = Buoy, 3 = Main rope, 4 = Rope, 5 = Thalus seaweed; A, B, C, D = Treatment; 1,2,3 = repetition.

At the conclusion of the research, cell counting was performed by sampling the seaweed thallus at five different locations: the base of the main thallus (PTU), the middle of the main thallus (TTU), the tip of the main thallus (UTU), the base of the new thallus (PTB), and the tip of the new thallus (UTB). Three fields of view were examined using a handheld tally counter under a microscope (Rozaki *et al*., 2013)

Agar content analysis was conducted at the Maros Brackish Water Aquaculture Research Institute Laboratory using the formula outlined by Jayasinghe & Pahalawattaarachchi (2016):

$$Agar\ Content\ (\%) = \frac{Agar\ Fiber\ Weight\ (g)}{Sample\ Dry\ Weight\ (g)} \times 100\ \%$$

As supporting data, physical and chemical parameters of the maintenance media water were measured. Temperature was recorded with a thermometer, dissolved oxygen was measured using a DO meter, salinity was assessed with a refractometer, and pH was determined using a pH meter. Additionally, ammonia, phosphate, nitrate, and nitrite levels were analyzed using a spectrophotometer (APHA, 2017).

Data on the rate of cell number and agar content were analyzed using analysis of variance (ANOVA), followed by the W-Tuckey further test (Aziza & Latif, 2024). Statistical tests were conducted using SPSS version 26.0.

RESULTS AND DISCUSSION

Analysis of cell number and agar content serves as a key parameter for evaluating productivity, growth activity, nutrient intake, and the chemical composition of seaweed (Massocato *et al*., 2022). The average number of cells observed during the research is presented in *Table* 2 and illustrated in Figure 2. The analysis of variance revealed that different plant spacing treatments had a significant effect on the number of cells ($p < 0.05$). Furthermore, Tukey's further tests indicated significant differences between treatments A, B, C, and D at the 95% confidence level.

The highest average number of cells was observed in treatment D (50 cm), with a value of 213.00 ± 9.64. This indicates that a planting distance of 50 cm is optimal for increasing the number of seaweed cells (Pong-Masak dan Sarira 2018). An optimal plant spacing allows each thallus sufficient space to develop, promotes effective water circulation around the thallus, facilitates nutrient and oxygen distribution, and aids in the removal of metabolic waste, thereby enhancing the health and productivity of the thallus (Jiksing *et al*., 2022).

Increasing plant spacing in each treatment was associated with a rise in the number of seaweed cells. Adequate spacing ensures that each seaweed seedling has access to sufficient sunlight (Creed et al., 2019), reduces physical pressure between thalli (Petrowski *et al.*, 2016) and minimizes the spread of pathogens and pests (Capdevila *et al*., 2018).

Conversely, the lowest cell count was recorded in treatment A (20 cm), with a value of 89.00 ± 2.00. This lower count is attributed to the closer plant spacing, which causes shading and reduces the light intensity received by the thallus. Insufficient light decreases the rate of photosynthesis, thereby reducing cell number. Additionally, close plant spacing hinders the flow of nutrient-rich water, limiting nutrient intake and inhibiting growth and cell division.

Cell count analysis is commonly employed in research because high cell counts signify ideal growth conditions, while low counts indicate stress or suboptimal conditions (Wakefield *et al*., 2022). The number of cells can reflect the photosynthetic efficiency of seaweed, as increased photosynthetic activity correlates with higher cell numbers (Takahashi, 2018). This analysis is also used to compare cell numbers across different strains or varieties to identify those better suited for growth and environmental adaptation (Rincent *et al*., 2019).

The average results of agar content during the research are presented in *Table* 3 and illustrated in Figure 3. Variance analysis indicated that different plant spacing treatments had a significant effect on agar content ($p < 0.05$). Tukey's further test revealed that treatments A and B did not significantly differ from each other but were significantly different from treatments C and D. Additionally, treatment C was significantly different from treatments A, B, and D, with all differences reaching significance at the 95% confidence level.

Based on *Table* 3 and Figure 3, the agar content varied across treatments, with values ranging from 11.54% to 14.29%. These results are relatively high and align with Rejeki *et al*., (2018), who reported an average agar content of 8% to 14% for *Gracillaria verrucosa*.

Table 2. Results of the Average Number of Cells During the Research

Treatment	Average Cell Number
A (20 cm)	89.00 ±2.00[a]
B (30 cm)	119.00 ±5.29[b]
C (40 cm)	173.00 ±2.65[c]
D (50 cm)	213.00 ±9.64[d]

Note: different letters indicate significant differences between treatments at the 5% level (p<0.05

Table 3. Results of Average Agar Content During the Research

Treatment	Average agar content (%)
A (20 cm)	15.59 ±0.47[a]
B (30 cm)	15.79 ± 0.68[a]
C (40 cm)	22.22 ±0.71[b]
D (50 cm)	27.31 ±0.69[c]

Note: different letters indicate significant differences between treatments at the 5% level (p<0.05)

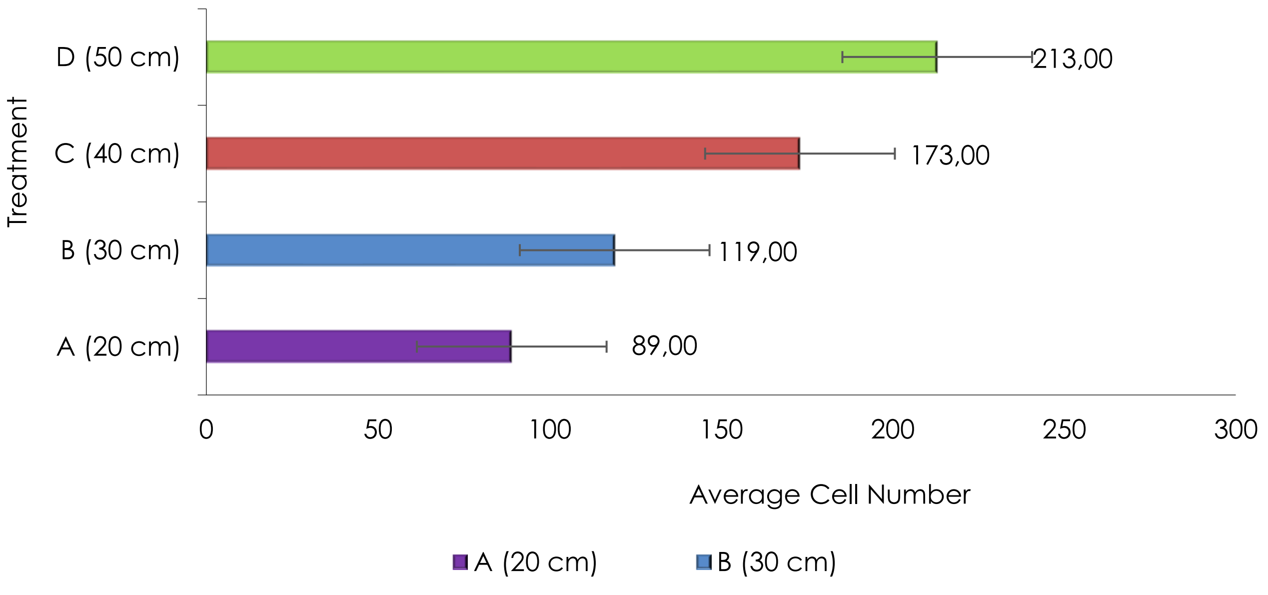

Figure 2. Results of the Average Number of Cells During the Research

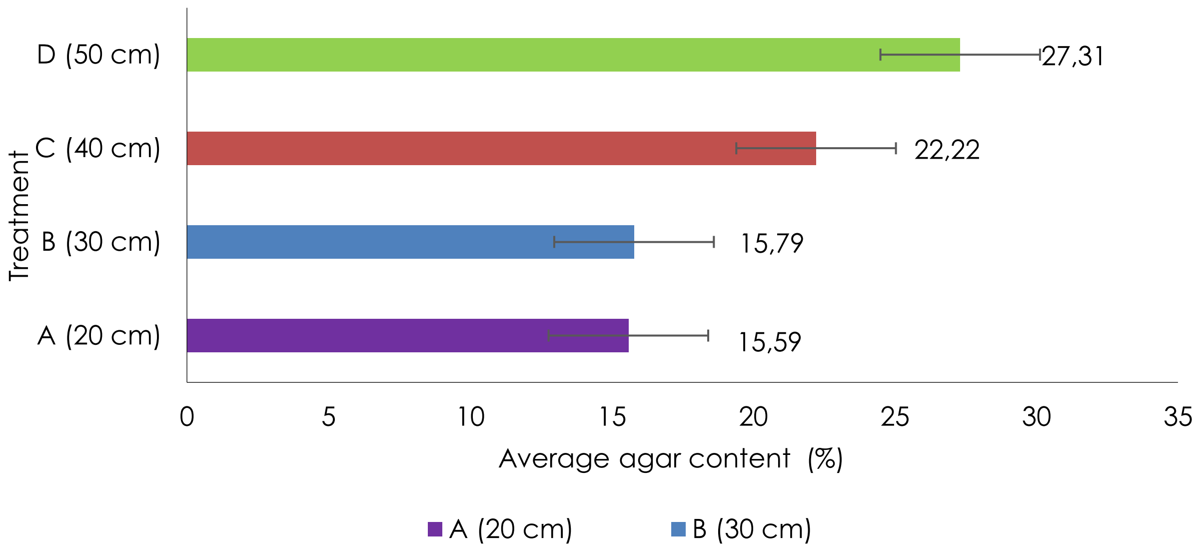

Figure 3. Results of Average Agar Content During the Research

The highest agar content, 27.31%, was observed in treatment D (50 cm). This increased agar content is attributed to more effective nutrient absorption, which is crucial for the formation of polysaccharide compounds such as agarose and agaropectin. These compounds are essential components of agar, with agarose making up 55–56% of the mixture (Budiyanti dan Musrif, 2019). This finding supports the research by Mulyaningrum & Suwoyo (2018), which indicates that the quality of *Gracillaria verrucosa* agar is influenced by water quality variables including phosphate, nitrate, salinity, and light penetration.

In contrast, treatment A (20 cm) had lower agar content due to reduced water flow carrying nutrients, limiting the availability of nutrients for each seaweed branch. This deficiency in nutrient absorption impairs the agar content (Vettori & Nikora, 2019). Closer planting distances, such as 20 cm, adversely affect the kinetic characteristics of nutrient absorption, resulting in lower rates of nitrate, ammonium, and phosphate uptake (Rees, 2003).

CONCLUSION

Planting distance significantly affects the number of cells and agar content in *Gracillaria verrucosa*. A planting distance of 50 cm resulted in the highest cell count, with an average of 213.00 ± 9.64 cells, and also yielded the highest agar content at 27.31%. Thus, a planting distance of 50 cm is the most optimal, producing both the highest number of cells and the greatest agar content.

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