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Growth Evaluation of the Seaweed *Kappaphycus striatus* F. Schmitz (Solieriaceae: Rhodophyta) with Mass Selection Method

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ABSTRACT

The suboptimal quality of seeds is identified as a contributing factor to the limited productivity observed in seaweed cultivation. A study was conducted to generate enhanced seaweed seeds through the utilization of the mass selection technique, as it offers a more straightforward and more accessible approach. From September 2022 to January 2023, the mass selection method was employed for seaweed cultivation in the aquatic environment of Tablolong Village, located in the region of East Nusa Tenggara. The growth and production of seaweed in each successive generation (G) exhibited superior performance when compared to both internal and external control groups. The observed daily growth rate of the chosen seaweed exhibited a consistent upward trend across successive generations, denoted as differences in treatment G1, G2, and G3. Specifically, the growth rates for these generations were recorded as 2.95±0.28, 3.55±0.29, and 5.58±0.25%/day, respectively. The absolute growth rates in G1, G2, and G3 were recorded as 2.60, 3.76, and 7.85g/ day, respectively. In the meantime, the production quantities per unit at G1, G2, and G3 were recorded as 3.89, 5.64, and 11.78kg/ unit, respectively.

INTRODUCTION

Seaweed is a photosynthetic organism that exhibits autotrophic characteristics, thriving by attaching itself to rigid surfaces like coral or by existing in a free-floating state within marine and coastal ecosystems. Countries possessing significant coastal potential face the formidable task of effectively and sustainably meeting the growing demand for seaweed resources on a substantial level. Additionally, seaweed can now be grown on land by using modified tank containers or fiber materials that are made to look like the water where it grows naturally. The global interest in seaweed cultivation has been steadily growing, driven by its recognized potential as a versatile resource for bioproducts, fuel production, and bioremediation purposes **(Zuldin *et al.*, 2016; Kumar *et al.*, 2023)**.

Indonesia holds a prominent position as a leading global producer of seaweed. In 2019, Indonesia witnessed a significant increase in the volume of seaweed production, reaching a staggering 9.66 million tons. This remarkable achievement accounted for approximately 38.7% of the global seaweed production, highlighting Indonesia's substantial contribution to this industry. Indonesia, following China, holds the position of the second-largest global producer of seaweed, thus establishing itself as a significant contributor to the "blue carbon" phenomenon **(Waters *et al.*, 2019)**. According to the **Indonesian Central Statistics Agency (2023)**, the primary contributors to production are the provinces of East Nusa Tenggara and South Sulawesi.

Medho and Muhamad (2017) asserted that the primary objective of seaweed management in East Nusa Tenggara, based on industrialization, is to enhance both the quality and productivity of seaweed production. The island region of East Nusa Tenggara Province exhibits considerable potential for the cultivation and extraction of marine resources, specifically seaweed. The province under consideration exhibits significant potential for the development of seaweed cultivation, encompassing an expansive area of 6,000 hectares. Notably, the total production of wet seaweed in this region attained a substantial volume of 2.2 million tons during the year 2020. The projected seaweed production for the year 2021 is expected to decline to a quantity of 1.4 million metric tons. The decline in seaweed production can be attributed primarily to factors such as substandard seed quality, inadequate maintenance practices, and improper post-harvest handling techniques. The primary hindrance to expediting seaweed production in the West Kupang District is the substandard quality of seeds **(Kusuma 2020; Kusuma *et al.*, 2021; Serihollo *et al.*, 2021; Amalo *et al.*, 2022; Kusuma *et al.*, 2022; Pratiwi *et al.*, 2023; Kusuma *et al.*, 2024)**. Seaweed farmers encounter the challenge of substandard cultivated seaweed seeds, primarily attributed to the sluggish growth of seaweed **(Pongmasak *et al.*, 2011; Porse & Rudolph 2017)**.

There is evidence suggesting a decline in the quality of seaweed seeds, characterized by relatively sluggish or stunted growth as well as susceptibility to ice-ice diseases. Genetic diversity decreases when seeds are used over and over again. This results in slower growth rates, less carrageenan production, and weaker gels. Additionally, this practice increases the vulnerability of the plants to extreme environmental fluctuations and diseases **(Pong-Masak & Parenrengi 2014; Fadilah *et al.*, 2016)**. If this issue remains unresolved in a timely manner, it is projected that it will adversely affect the future production of seaweed. Modern technology makes it easier to get better seeds for farming, and these seeds are easy to use and can adapt to different conditions. This can help increase production and growth rates. High-quality seaweed seeds can be obtained in a number of ways, such as through the variety selection method (also known as "*mass selection*"), tissue culture techniques, and genetic engineering. Methods such as tissue culture and genetic engineering represent medium- and long-term strategies, whereas mass selection serves as a short-term strategy that offers practicality and ease of adoption for cultivators directly in the field. This method does not necessitate specific infrastructure or specialized human resources, resulting in cost-effective inputs. In comparison to alternative methodologies, mass selection is a highly effective method for rapidly generating superior seed varieties that exhibit resistance to both diseases and environmental conditions. Additionally, this approach offers distinct advantages that are specific to particular locations or regions. Mass selection programs can effectively utilize early varieties and seed sources, particularly those that have been locally adapted over an extended period within a specific region **(Maros, 2011)**.

The seaweed seeds obtained through the process of variety selection exhibited a more favorable daily growth rate response. In comparison to both the internal control and external control groups, production levels increased by 15–25% **(Pong-Masak *et al.*, 2013)**. Additionally, the daily growth rate was measured at 2.92%/day, while the control group exhibited a growth rate of 1.58%/day **(Parenrengi *et al.*, 2016)**. A study was conducted with the aim of identifying seaweed cultivars characterized by rapid growth and the ability to enhance seaweed production by utilizing seaweed seeds derived from carefully selected varieties. The anticipated outcome of this study is that its findings will furnish valuable insights and facilitate future investigations aimed at delving more comprehensively into the mass selection technique with the objective of generating enhanced seaweed seeds.

MATERIALS AND METHODS

Research sites

The experiment was carried out in Tablolong Village, West Kupang District, Kupang Regency, East Nusa Tenggara Province, situated at coordinates 10°18′ 33"S and 123°28′07"E during the period from September 2022 to January 2023.

Seaweed cultivation construction

The present study focused on the fabrication of cultivation containers designed for mass selection employing the long-line method. The dimensions of the seaweed cultivation unit employed are 5 x 10m. The span ropes were spaced at a distance of 1m, while the clumps within each span were positioned 20cm apart. Therefore, within a single seaweed cultivation enterprise, the number of clumps was limited to a maximum of 250. The weight of the seeds that were planted exhibited a range of 50–54g, while the maintenance cycle spaned a duration of 30 days. The uniform spacing of the clump ropes ensured an adequate room for movement and an equitable distribution of nutrients to facilitate the optimal growth of seaweed. The act of sowing seeds was conducted at a depth of 30cm below the water's surface. The removal of seaweed from pest organisms was conducted on an as-needed basis (Fig. 1).

Supply of initial seed stock

The initial stock population of *Kappaphycus striatus* seeds originated from distinct cultivation sites, which were established through the efforts of local farmers engaged in cultivation practices. The initial seeds were chosen according to particular parameters. These criteria included the seaweed's age, which ranged between 25–30 days. Additionally, the thallus shape was proportional to its size and length. Furthermore, the condition of the thallus was assessed based on its health, freshness, cleanliness, bright coloration, abundance of branches, and its lush and spiky appearance.

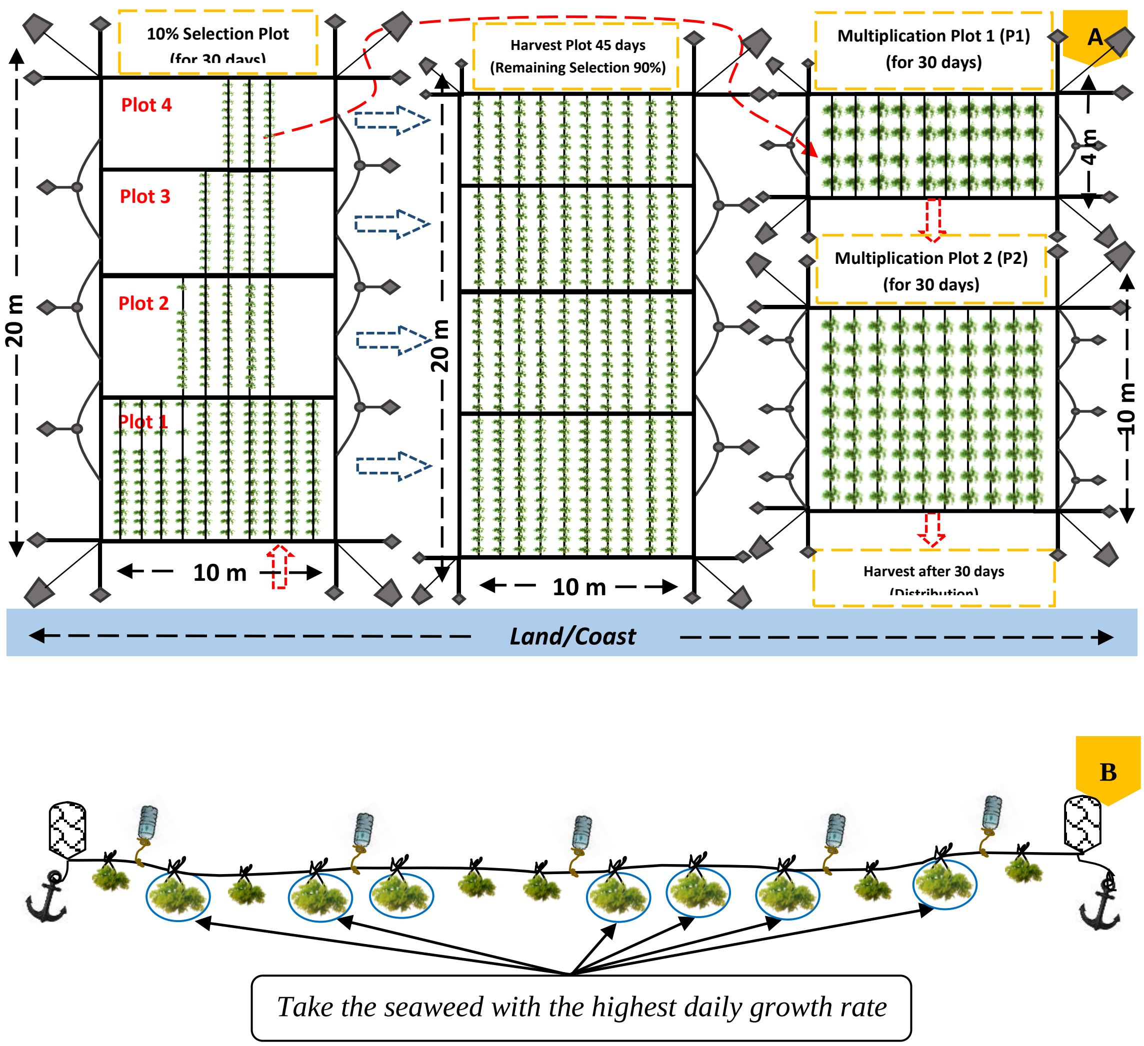

Fig. 1. Seaweed cultivation construction. **(A)** Top view; **(B)** Side view

Monitoring of water quality

The water quality parameters were collected during the time frame of 08:00 to 10:00 am on each sampling day, with a frequency of once every 30 days. A thermometer was employed for the purpose of quantifying the temperature of water, typically denoted in degrees Celcius (°C). The water current speed (m/s) was measured using a flow meter (Flowatch, FL-03), while the water brightness (cm) was assessed using a Secchi disk tool. Dissolved oxygen concentrations (mg/l) were measured using a dissolved oxygen meter (DO-5509), while pH levels were measured using a pH meter (HANNA instrument, HI 98107). The concentrations of orthophosphate (mg/l) and nitrate (mg/l) were analyzed at the East Nusa Tenggara Province Environmental and Forestry Service Laboratory.

Seaweed selection

Seaweed that has been grown for 30 days as a new broodstock population (G0) was then used as the main material for seaweed selection. The selection procedure employed in this study was based on the methodology developed by **Pong-Masak *et al.* (2011)**, which utilized a selection indicator in the form of the daily growth rate (DGR). The process of selection involved identifying the top 10% of DGR values within each maintenance cycle. The remaining values, referred to as rejects or the cut-off, were then utilized as seeds for internal controls in research.

Harvesting

The harvesting of specifically chosen seaweed occurs once the seaweed reaches an age of 42 days, which contrasts with the harvesting conducted during general selection activities, which takes place at 30 days. The individual strands of seaweed are systematically extracted from the bundles and subsequently placed into seaweed baskets for the purpose of undergoing sun-drying, until the moisture content reaches a maximum threshold of 35%. Seaweed baskets are artisanal products crafted by the spouses of cultivators residing in cultivation sites **(Pratiwi *et al.*, 2023)**.

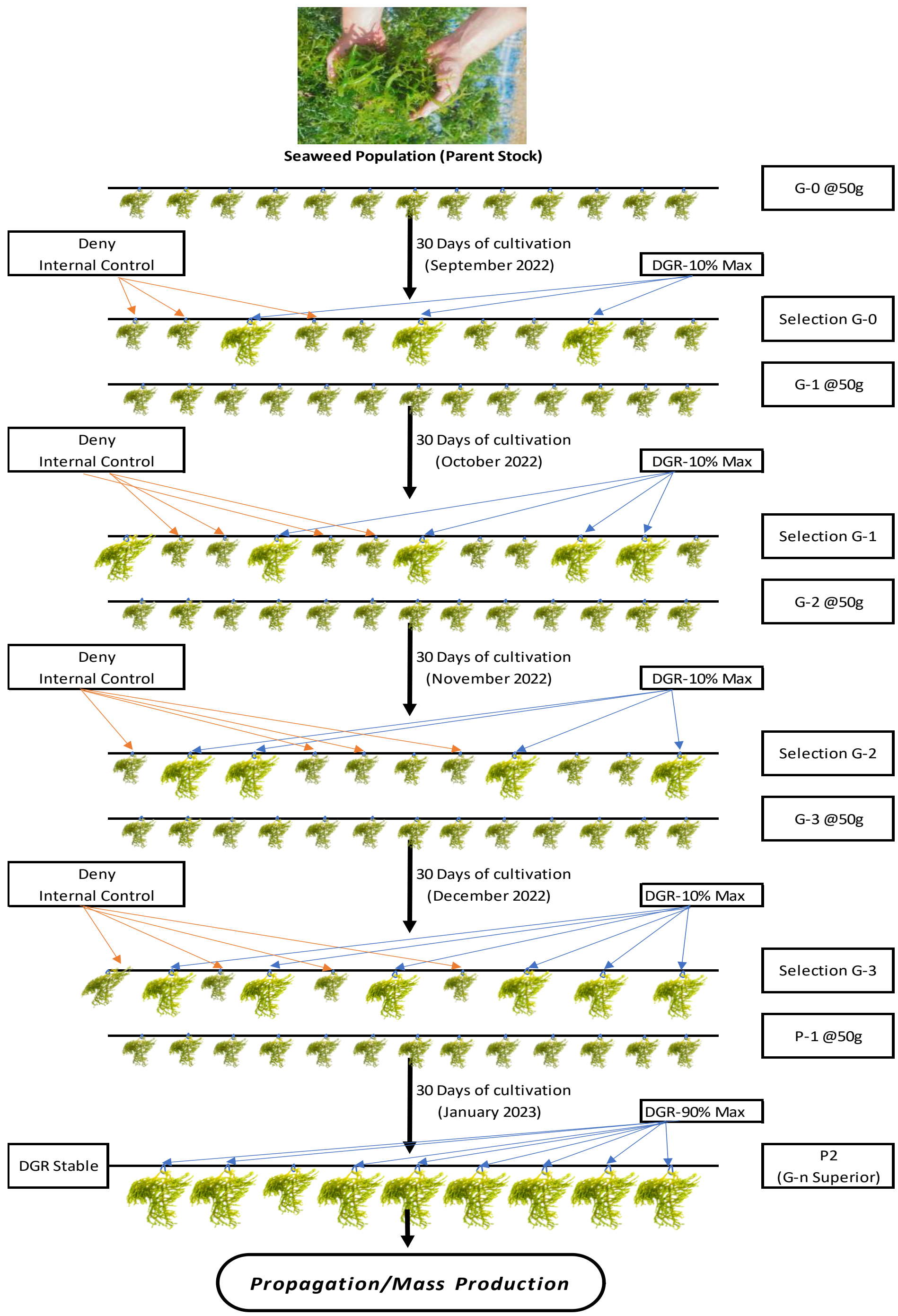

Fig. 2. Scheme of mass selection stages for *Kappaphycus striatus*

Seaweed monitoring

Data were collected at regular intervals of four weeks by selecting a representative sample of seaweed, specifically the top 10% with the highest daily growth rate, from each section of rope. The seaweed was subsequently measured using a digital scale that has a precision of 0.01g. Based on previous research, daily growth rate (%/day) was calculated **(Yong *et al.*, 2013)**, as follows: (*ln*Wt–*ln*Wo/t) x 100%, absolute growth rate (g/day) was calculated **(Wong *et al.*, 2014)**, as follows: (Wt–Wo)/t, and seaweed production (kg/m^2) was calculated **(Lugert *et al.*, 2016)**, as follows: (Wf–Wi)/A. Where *ln* is the natural logarithm, *Wt* is the final fresh weight (g) on day *t*, *Wo* is the initial fresh weight (g), *t* is the number of days of maintenance, *Wf* is the weight at harvest (kg), *Wi* is the initial weight (kg), and *A* is the cultivation surface area (m^2).

Statistical analysis

Parametric analysis was used to check if the seaweed growth and production data were normal (using the Shapiro-Wilk test) and if the variance was the same for all the samples (using the Levene test). The data were presented in the form of the minimum mean ± standard deviation (maximum) within the framework of a completely randomized design (CRD) model. To look for big differences in growth rate and seaweed production, the study used a two-way analysis of variance (ANOVA) with a 95% confidence level ($P < 0.05$) and the Duncan multiple range test (DMRT) post hoc test.

RESULTS AND DISCUSSION

Daily growth rate (DGR)

The growth rate of seaweed during three cultivation cycles is depicted in Fig. (3). The selection results had the highest average DGR at 4.03±1.38%/day, while the internal control had a DGR of 2.94±1.12%/day and the external control had a DGR of 2.65±0.85%/day. These three populations were watched over the course of the cultivation cycle. The utilization of selection techniques in the cultivation of *Kappaphycus striatus* seaweed seeds has been observed to enhance growth rates in comparison to non-selected seeds. **Parenrengi *et al.* (2016)** conducted a study that corroborated previous research findings. Specifically, their investigation demonstrated that the DGR of seaweed was significantly higher in selected seedlings, ranging from 2.26–3.47%/day, compared to the control group with DGR values ranging from 1.13–1.82%/day. This finding was also observed during the implementation of seaweed and selection, wherein a daily growth response exceeding 5% or a production increase of 32–40% was demonstrated in comparison to the control group.

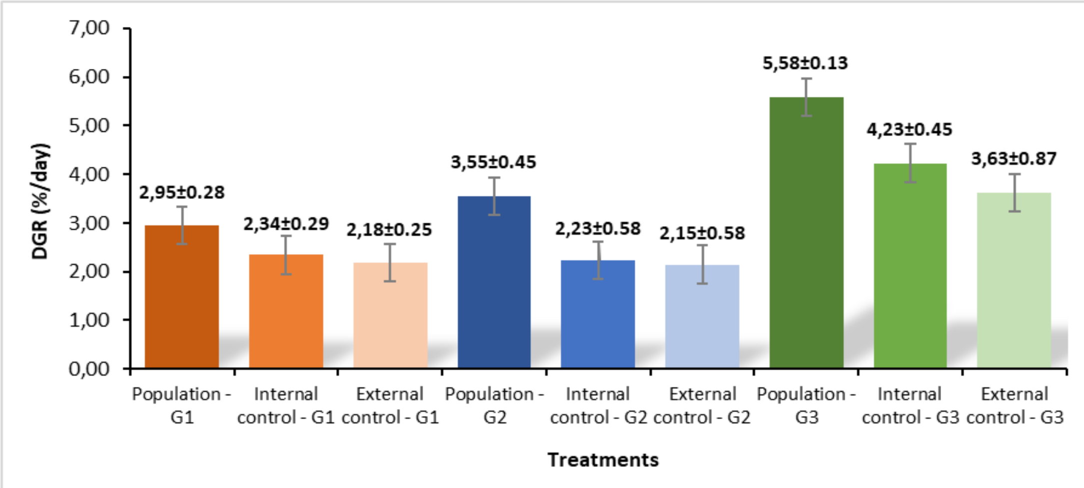

Fig. 3. Daily growth rate of seaweed during three cultivation cycles

Furthermore, DGR response exhibited a higher value of 5.81%/day, in contrast to the internal control, which displayed a DGR of 3.87%/day **(Pong-Masak *et al.*, 2014; Pong-Masak & Priono 2015)**. **Pong-Masak and Sarira (2018)** conducted a study in which they observed a significantly higher DGR in selected seaweed seeds compared to a control group ($P< 0.05$). The selected group exhibited a DGR of 5.87%/day, whereas the control group had a DGR of 1.89%/day. The average DGR of selection results was found to be 5.53%/day, based on data collected from three seed production cycles (G1–G3). In comparison, the control group exhibited an average DGR of 2.19%/day. Hence, it can be inferred that the implementation of the seaweed selection method yields a significant enhancement in the daily growth rate. Due to the seaweed population in this generation being the outcome of superior seed selection from the two prior populations, the G3 treatment yielded the greatest DGR value.

Absolute growth rate (AGR)

Based on the observations made of the seaweed's absolute growth over three maintenance cycles, it is clear that there was a significant and noteworthy absolute growth response. Specifically, the growth rate ranged from 2.60–7.85g/ day, resulting in a growth range of 131.50–289.06g/ clump over the course of the study period. The duration of the maintenance life cycle is 30 days. According to the data presented in Fig. (4), it can be observed that seaweed production was highest in the G3 population, with a recorded value of 7.85g. Following this, the G2 population exhibited a lower seaweed production rate of 3.76g/ day. Finally, the G1 population displayed the lowest seaweed production, with a recorded value of 2.60g/ day. The findings from the analysis of variance indicate that the selection of seaweed from different populations, as well as the control group, significantly impacted the absolute growth of seaweed.

Furthermore, the absolute growth sampling findings for each clump demonstrate a low difference in comparison with the values obtained from the daily growth rate sampling data (Fig. 4). The correlation between the daily growth rate and the absolute growth of seaweed is dependent on the prevailing circumstances of the aquatic environment at the culture site.

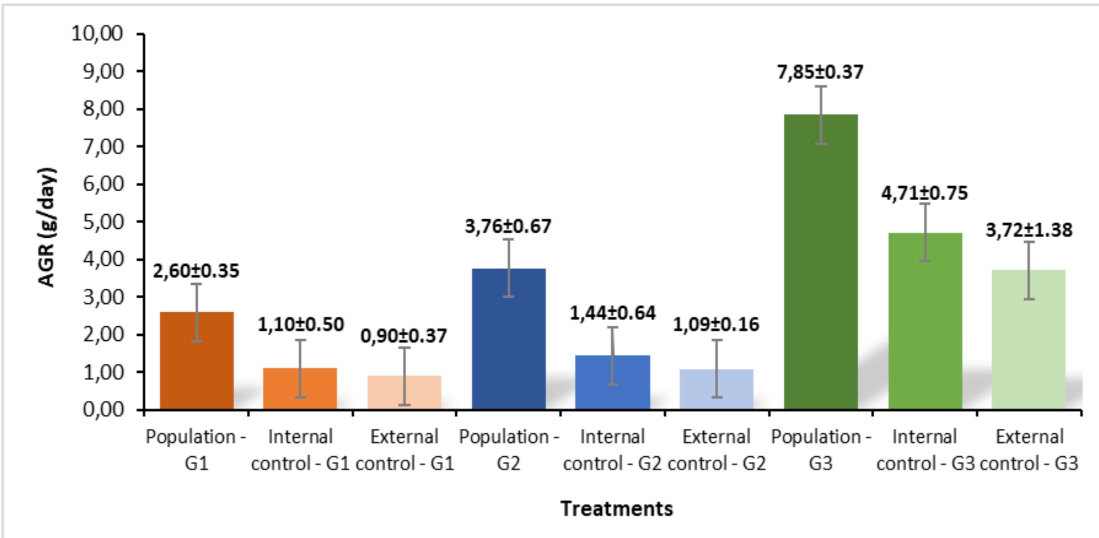

Fig. 4. Absolute growth rate of seaweed during three cultivation cycles

The variability in meteorological circumstances exerts a substantial influence on the modifications in water quality over the course of the agricultural cycle. The alterations in water quality have a subsequent impact on the availability of nutrients, thereby increasing the susceptibility to insect and disease infestations. The observed augmentation in seaweed biomass during the maintenance period demonstrates a level of growth that satisfies or surpasses pre-established parameters. As a result of this selection process, the growth of the seeds has been subjected to a clump selection approach that is generated from the collection of seaweed, ensuring optimal performance. The approach of mass selection has led to a notable rise in the population of seaweed, which in turn has had a beneficial impact on productivity. Various species of seaweeds possess beneficial genetic material and derivatives of a compound known as "growth hormone," which aid in the cultivation of high-quality seedlings. **Pong-Masak and Simatupang (2017)** asserted that genes are of paramount importance in the inheritance of genetic traits observed across various species.

Seaweed production

Based on the information in Fig. (5), the G3 population was able to produce 11.78kg/ unit of time through the selection process. Similarly, the G2 population exhibited a lower seaweed production of 5.64kg/ unit, while the G1 population had the lowest production rate at 3.89kg/ unit. It was found that the seaweed from each population and its controls had a big effect on the growth of seaweed through an analysis of variance.

Seeds of seaweed that possess advantageous genetic features demonstrate increased adaptation to variations in the environment, heightened resistance to illnesses and a greater ability to absorb nutrients from their surroundings. In the study conducted by **Fadilah *et al.* (2016)**, it was noted that *Kappaphycus* sp., which was chosen for investigation, demonstrated a greater rate of development and a 15.52% elevation in plant growth hormones, notably kinetin, in comparison to the internal control seaweed.

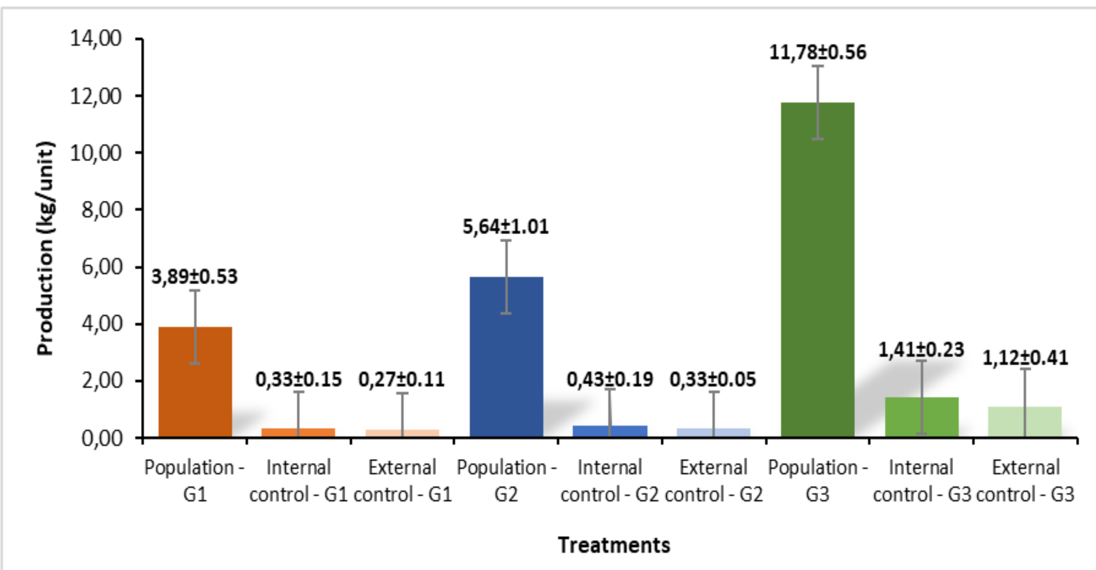

Fig. 5. Production of seaweed during three cultivation cycles

The statistical method referred to as analysis of variance (ANOVA) demonstrates that the growth of *Kappaphycus striatus* is considerably affected by the seaweed samples obtained from various populations, as well as the control group. The environmental conditions of the seas where cultivation was carried out had a significant impact on the growth of seaweed during the study. It is critical to use the seasonal calendar as a guide in deciding the type of variety and the best period for seaweed production. According to **Orbita (2013)**, the maximum seaweed growth occurs during the "habagat" season, which is characterized by moderately powerful waves, low temperatures, and low salinity, followed by rain. Since the seaweed population in this generation was selected from the two preceding populations' best seeds, the G3 treatment yielded the maximum value of seaweed production.

Water quality

Water temperature at the research site exhibits a range of 28–32°C. **Pong-Masak *et al.* (2013)** stated that the selection site for superior seed types of *Kappaphycus* sp. necessitates an optimal temperature range of 26–29°C. In addition, the activity of enzymes involved in the biochemical processes of respiration and the dark reactions of photosynthesis is influenced by temperature **(Rongbin *et al.*, 2013)**.

During the investigation, the pH of the waters was 8.0–8.5, which encouraged the growth and development of *Kappaphhycus striatus*. The pH concentration in water is inversely associated with the rate of photosynthesis, which rises with lowering pH and decreases with increasing pH above 8.5 **(Iris *et al.*, 2016)**. Since each *Kappaphycus* sp. variation responds differently to changes in physicochemical parameters, a study is required to determine the best circumstances for each variety **(Yunque *et al.*, 2011)**.

The salinity level of the waters at the study site is 32–34ppt. *Kappaphycus* sp. is a seaweed that will not tolerate high salinities, preferring salinities between 28 and 35ppt **(Sudradjat, 2008; Asni 2015)**. However, an optimal salinity of 32–34ppt is required for the selection location for excellent seed types of *Kappaphycus* sp., according to **Pong-Masak *et al.* (2013)**. Salinity is one of the water quality characteristics that controls the energy-intensive osmoregulation process of aquatic organisms. **Ariosandi *et al.* (2011)** say that osmoregulation in cells is linked to how saltiness affects seaweed's growth, thallus formation, and new shape development.

During the investigation, the current speed varied from 0.25-0.33m/ s. The best current speed for developing *Kappaphycus* sp. is 0.2–0.4mg/ s, as noted by **Pong-Masak *et al.* (2013)**. If the speed of weak ocean currents affects the amount of biofouling in seaweed, then the speed of the currents is related to the amount of biofouling in the thallus. Aside from improving the flow of nutrients, strong currents also keep pathogens from sticking to the seaweed thallus's surface **(Fadilah, 2014)**. The movement of water masses in Indonesian waterways differs between the rainy and dry seasons, resulting in variations in hydrological circumstances that influence high and low levels of water production **(Maryunus, 2018)**.

The orthophosphate content in the waters throughout the research was 0.01mg/ l, which is lower than in other seasons and sites for seaweed farming. This is supposed to be the reason for Tablolong Village's subpar seaweed growth. According to **Akmal *et al.* (2017)**, the orthophosphate suitability value for seaweed is in the range of 0.064–0.599mg/ l, but according to **Pariakan *et al.* (2019)**, an orthophosphate concentration of 0.37–0.5 is good for growth. *Kappaphycus* sp. has a competitive advantage under brief periods of low phosphorus, according to **Boyd and Tucker (2012)**. This is due to the fact that when the waters are nutrient-rich, seaweed will take phosphorus intracellularly, digest it, and store phosphorus reserves.

During the investigation, nitrate concentrations were low, ranging from 0.001–0.025mg/ l. This is impacted by temperature and salinity, with nitrate being difficult to dissolve in water when the temperature varies between 28–32°C and the salinity is between 32–34ppt. According to **Boyd and Tucker (2012)**, when temperature and salinity rise, so does the equilibrium concentration of dissolved nitrogen. Nitrate is a nutrient required for seaweed development and, like phosphorus, is a limiting element. The ideal nitrate content in waterways for seaweed development, according to **Mustafa *et al.* (2017)**, is 0.9–3.2mg/ l.

CONCLUSION

Seaweed seeds obtained by mass selection grew and produced more than seeds obtained from local farmers. Selection enhanced the daily growth rate of *Kappaphycus striatus* in populations G1, G2, and G3. The G3 population has a substantially greater daily growth rate value than the internal and external controls. The absolute growth response of chosen seaweed over three raising cycles was quite strong during a 30 day rearing period. Aside from that, the G3 population selection results showed the greatest seaweed production.

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