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Selection and cultivation of seaweed *Kappaphycus alvarezii* using a long line system (outdoor)

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ABSTRACT

In outdoor cultivation at Amal Beach, three seeding mass selections were reared for 30 days for each cycle. Each variety had a different DGR %/day (Daily Growth Rate %/day) value at the end of the growing season on G1-G2-G3 ranging from 3.62-5.90 %/day, which were still categorized as good, and G3 var.Lampung had the highest DGR value. Carrageenan yield values in this study, G1 to G3, ranged from 23.16% to 45.87%, where the G3 > G2 > G1 treatment. The best thallus samples (20% were selected for each generation) resulted in carrageenan yield > sample population in each variety, where var.Lampung > var.Tarakan > var.Kupang. The biplot analysis shows that the DGR variable vector coincides with the IAA, Phosphate, Gibberellin, and Ammonia vectors. Ammonia stepwise regression analysis (sig<α) affects DGR with an Ammonia coefficient value of 4.89; meaning that for each increase in the Ammonia value by one unit, the estimated mean value of DGR will increase by 4.89. Thus, the application of the *mass* based on the results of this study was able to increase the DGR value *Kappaphycus alvarezii,* which in turn produce the highest yield of carrageenan with *K. alvarezii* var.Lampung G3 was the best seed candidate to be developed at the seaweed cultivation site in Tarakan City.

Introduction

Seaweed *Kappaphycus alvarezii* is a leading commodity in Indonesia's fisheries revitalization program. This seaweed produces carrageenan, which has significant economic value for industry and trade as a raw material for food, cosmetics, and medicines. High production demands *K. alvarezii* requires certainty of superior seeds that multiply, are widely available, and are of good quality. Seaweed seeds that are continuously used without clear genetic quality can cause a decrease in rate and slow growth and are susceptible to pest and disease attacks and even death which results in crop failure. Selection is one of several methods that can increase the daily growth rate of seaweed *K. alvarezii* quickly, cheaply, easily, massively, and sustainably to meet the availability of superior seeds (Pongmasak *et al.*, 2018).

Selection can be interpreted as selecting best specific individuals involved in producing the continuation of the next generation (Noor, 2004; Satriani *et al.,* 2011). Selection is expected to increase the frequency of the desired potential genes and reduce the frequency of unwanted genes that are potentially negative for the continuity of generations (Moose & Mumm, 2008).

Mass selection is a quick way to get cultivars that adapt broadly to new environments. This method is effective (Qosim, 2008) in obtaining particular adaptations to extreme environmental pressures such as high/low pH, dryness, cold, heat, disease, etc. Plants with high resistance are selected to form cultivars adapted to extreme environments and plant populations that are genetically heterogeneous for mass selection by planting with irregular spacing or tight spacing (Sumardi, 2020).

Challenges in breeding efforts include narrowed the population (population bottleneck) and increasing inbreeding, decreasing heterozygosity, loss

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of essential alleles, decreasing fitness (vitality), and the founder effect (forming a new population with less genetic diversity than the initial population) (Twumasi *et al.,* 2014). The selection process must consider the standards and benchmarks that farmers/cultivators are interested in and can be doubted for each selected phenotype (Qosim, 2018). In seaweed, determination of the characters selected must represent traits of high economic importance such as growth and carrageenan content (Leliaert *et al.*, 2014; Akbar *et al.*, 2023).

Traits elite from cultivated seaweed illustrates a reduction in genetic diversity, in essential alleles, and in heterozygosity which results in low adaptability and low selection opportunities (Roleda *et al.,* 2021). In addition, the use of the same seed source continuously results in a decrease in genetic diversity, making it susceptible to environmental changes; low resistance of cultivated seaweed to disease (for example, ice-ice) and due to a decrease in genetic diversity causes the potential fitness against selection to be low (Parenrengi *et al.,* 2016). A controllable treatment must accompany the validity of the trait to produce an elite trait (Ekroth *et al.,* 2019). One of the efforts to overcome this problem is to conduct breeding selection supported by applying molecular markers in mass selection (Porse *et al.,* 2017). This study aimed to evaluate mass selection from generation to generation based on the growth (DGR), carrageenan quality, and phytohormone.

Materials and Methods

Location and time of research

This research was conducted at Amal Beach, Tarakan City, Kaltara (Figure 1). Mass selection for seed cultivation was carried out for 30 days per cycle for each generation from G-0 to G-3 until a stable DGR value was obtained. This research was conducted from March-June 2021.

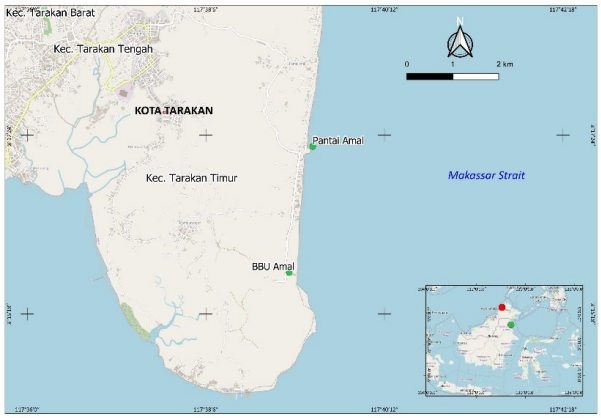


Figure 1. Amal Beach, Tarakan City, Indonesia (117°39'50.1624"E-3°15'53.03399"N)

Mass Selection Procedure

Seaweed *K. alvarezii* used in this study refers to the mass selection protocol (Figure 2) for seaweed developed by Pongmasak *et al.* (2011). The method was modified using 3 stocks of *K. alvarezii* based on the DNA sequencing analysis results, which we stored in the NCBI GenBank database. These three varieties are then cultivated for up to 3 generations or until the DGR value is stable with a long line at Amal Beach, Tarakan.

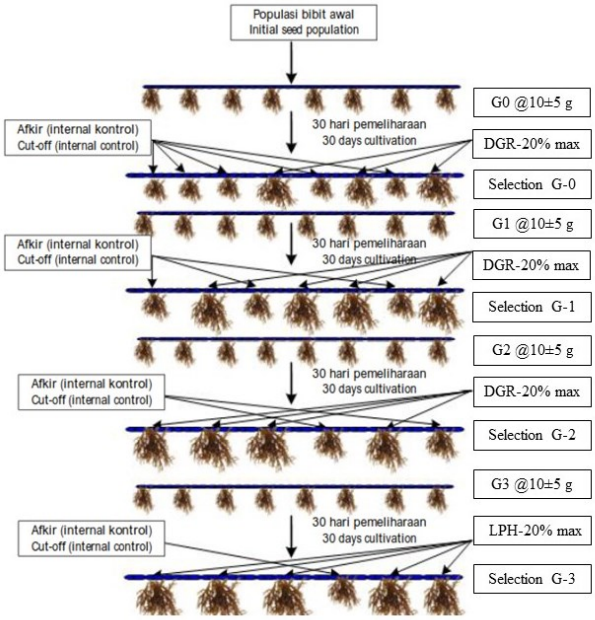


Figure 2. Seaweed selection procedure based on daily growth rate (DGR) parameter (adoption of illustration from Pongmasak *et al.* 2011)

In selecting seaweed varieties/strains in this study, seed production in each generation was carried out through a vegetative propagation process to obtain the next generation, known as the seed production cycle (Pongmasak *et al.*, 2011). Heritability is determined based on mass selection's average difference between the selected DGR and the control population. The selection response is estimated based on the increase in the selection DGR compared to the internal control DGR.

Selection activities with the highest cut-off of 20% were DGR selections (Fadilah *et al.*, 2016). Cultivation was carried out outdoors using a long line system (Figure 3) for 30 days for each seaweed cultivation cycle. Seaweed seedlings were planted on span rope media, with eight wooden stakes instead of anchors and 1500 ml plastic bottles as buoys. On the span rope, 100 points, and at each point, 10 ± 5 grams of seaweed seeds were tied (Rama *et al.,* 2018) with a spacing between the ropes of 15 cm.

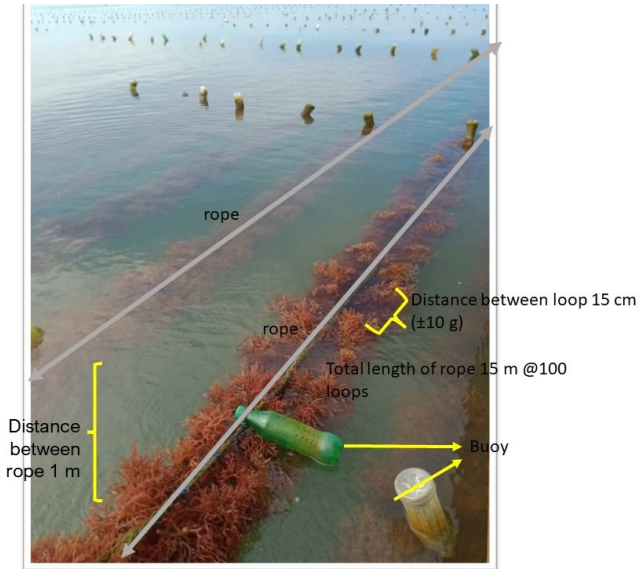


Figure 3. The long line at a particular point

Observation Parameters

Seeds of *K. alvarezii* for mass selection activities, namely seaweed, were obtained from sequencing DNA analysis available in GenBank NCBI (Accession version *K. alvarezii* var.Lampung OP136980.1, var.Tarakan OP136987.1, and var.Kupang OP136977.1). The selected seedlings were 30 days old and had healthy thallus, were clean, fresh, brightly colored, had many branches, and were lush. Before selection, the seedlings were adapted at the study site for a month at BBU Amal indoor system (Satriani *et al.,* 2022). The seeds from mass selection were used for mass production activities for three mass selections. Each cycle is symbolized with G0, G1, G2, and G3. In G3, it is expected that DGR has stabilized.

Measurement of physiological characteristics and water quality is carried out in each cycle of mass production. The test variables observed in each seaweed clump include the daily growth rate %/day (DGR %/day) calculated using the initial weight value (W0) and the biomass obtained over a specific time (Wt), using the formula DGR %/day = ((ln Wt – ln Wo)/t) x 100) (Hurtado *et al.,* 2017). Carrageenan yield includes the yield content, moisture content, thallus moisture content, thallus ash content, and silicate (acid insoluble ash content) (BRKP, 2003). Total sugar content was calculated using the Luff Schoorl method (SNI), phytohormones from *K. alvarezii* in the form of IAA, gibberellin, zeatin, and kinetin preparation were used, followed by measurements using HPLC (Linskens & Jackson, 1987). Supporting data is in the form of water quality parameters, namely the physical and chemical values of the waters, including brightness, salinity, temperature, pH, ammonia, nitrate, nitrite, and phosphate, which are measured periodically.

Data analysis

Physiological characteristics data were analyzed by analysis of variance (ANOVA) at a 5% test level using SPSS 22. If there is an effect, then the analysis is continued using the BNT test. Stepwise Biplot analysis is used to evaluate the relationship between variables. The values of water quality data are analyzed descriptively.

Results

Seaweed was cultivated outdoors at Amal Beach for three seeding mass selections (each cycle with a rearing time of 30 days). Each variety had a different DGR value at the end of the growing season in G1-G2-G3 (Table 1).

Table 1. DGR Value *of K. alvarezii* at Amal Beach Outdoor Cultivation.

No.	Variety P-value 0.000	Intensity of selection (IS)	n-th Generation	Value DGR (%/day)	Weight average (μ) gram	Standard Error (SE)
1.	Lampung	1.51	G1 (29 stretch of rope)	4.02	45.60±26.54	0.49
2.	Lampung	2.05	G2 (18 stretch of rope)	4.74	46.20±21.08	0.50
3.	Lampung	1.70	G3 (12 stretch of rope)	5.90	60.14±35.63	1.03
4.	Tarakan	1.81	G1 (50 stretch of rope)	3.67	31.68±18.65	0.26
5.	Tarakan	3.09	G2 (26 stretch of rope)	4.11	37.41±20.98	0.41
6.	Tarakan	1.20	G3 (18 stretch of rope)	5.22	48.29±25.35	0.60
7.	Kupang	1.52	G1 (36 stretch of rope)	4.46	40.32±14.93	0.25
8.	Kupang	1.58	G2 (36 stretch of rope)	3.62	32.62±16.80	0.28
9.	Kupang	2.00	G3 (25 stretch of rope)	4.84	43.91±18.46	0.37
10.	Internal control 1 (Lampung)	-	G2 (11 stretch of rope)	4.24	35.72±20.78	0.63
11.	Internal control 2 (Lampung)	-	G3 (6 stretch of rope)	3.14	27.75±13.50	0.55

Note: One stretch of rope contains 100 clumps of seeds cultivated for 30 days.

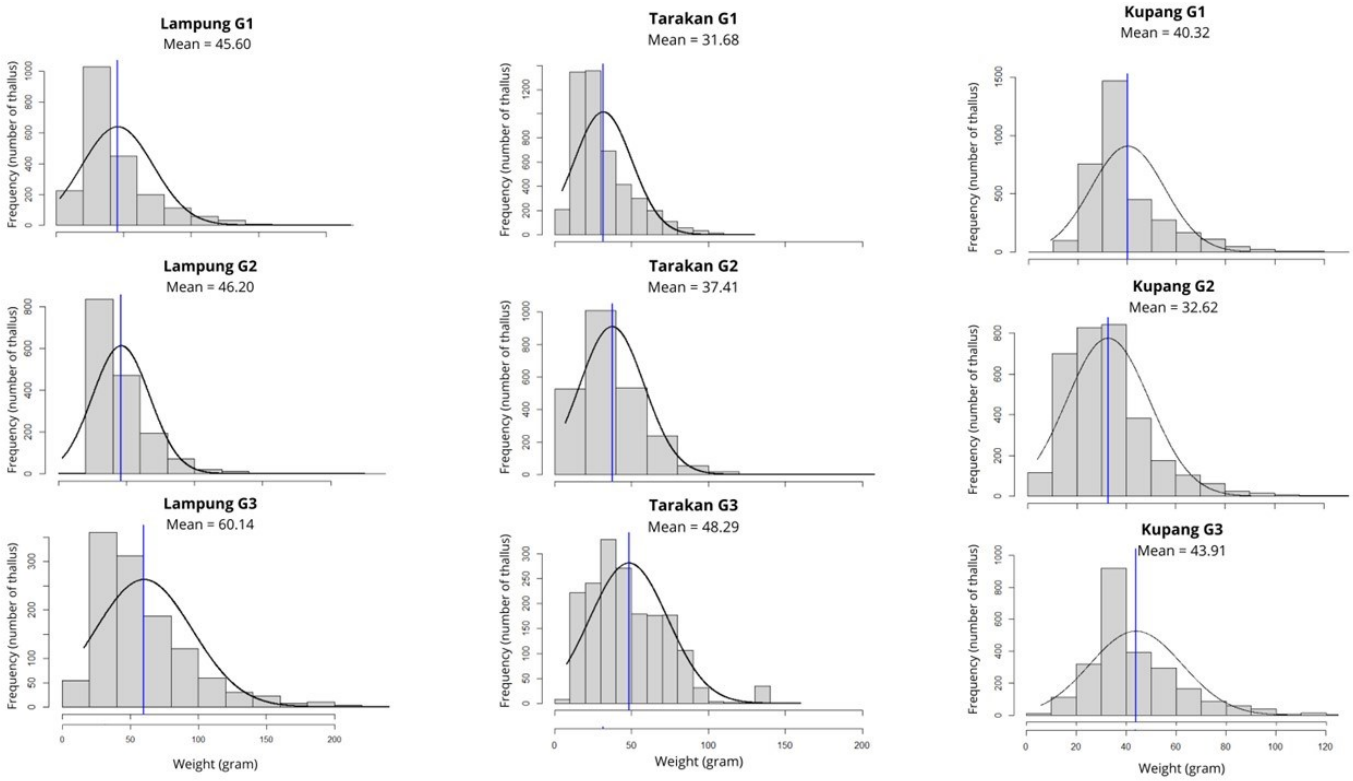


Figure 4. Response to selection *K. alvarezii* of different varieties (Kupang, Lampung, Tarakan) to the average harvest weight produced in each cycle (G1, G2, G1)

Table 2. Carrageenan Value

Treatment P-value 0.000	Variable	Seed of *K. alvarezii* Varieties					
		Lampung (n=3)		Tarakan (n=3)		Kupang (n=3)	
		Carrageenan Value	S.E	Carrageenan Value	S.E	Carrageenan Value	S.E
Non selected	Dry seaweed moisture content (%)	12.41±0.72[a]	0.42	10.89±1.35[a]	0.78	14.72±0.15[b]	0.09
	Water content of carrageenan yield (%)	14.34±0.06[b]	0.03	13.83±0.25[ab]	0.14	13.33±0.45[a]	0.26
	Carrageenan yield (%)	44.71±1.19[a]	0.69	36.73±7.53[a]	4.35	43.87±4.58[a]	2.64
	Viscosity (cP)	775±5[c]	2.89	555+5[a]	2.89	638.33+7.63[b]	4.41
The best of G1	Dry seaweed moisture content (%)	36.50±2.60[c]	1.50	16.40±0.60[a]	0.35	19.13±0.06[b]	0.03
	Water content of carrageenan yield (%)	33.74±5.00[c]	2.89	6.09±0.50[ab]	0.29	13.98±7.84[b]	4.53
	Carrageenan yield (%)	40.66±4.60[b]	2.66	39.87±3.26[b]	1.88	23.65±8.64[a]	4.99
	Viscosity (cP)	910±5[d]	2.89	895.83±7.2[d]	4.16	700±12.5[c]	7.22
G1 population	Dry seaweed moisture content (%)	14.50±1.60[a]	0.92	15.50±0.80[a]	0.46	14.90±1.70[a]	0.98
	Water content of carrageenan yield (%)	10.79±6.31[ab]	3.64	2.97±1.41[a]	0.81	4.36±0.10[a]	0.06
	Carrageenan yield (%)	27.56±7.50[a]	4.33	27.72±3.14[a]	1.81	23.16±5.81[a]	3.35
	Viscosity (cP)	625±108.97[bc]	62.91	579.17±50.52[ab]	29.17	508.33±19.09[a]	11.02

The best of G2	Dry seaweed moisture content (%)	20.53±4.79[d]	2.77	15.60±1.39[c]	0.80	7.53±0.23[ab]	0.13
	Water content of carrageenan yield (%)	12.59±0.32[a]	0.18	12.70±0.28[ab]	0.16	13.27±0.25[b]	0.14
	Carrageenan yield (%)	44.50±5.22[d]	3.01	34.27±2.48[ab]	1.43	31.33±0.59[ab]	0.34
	Viscosity (cP)	925±12.5[d]	7.22	712.50±87.5[c]	50.52	754.17±7.22[c]	4.17
G2 population	Dry seaweed moisture content (%)	4.20±0.00[a]	0	6.00±1.00[a]	0.58	10.93±0.58[b]	0.33
	Water content of carrageenan yield (%)	13.91±0.47[c]	0.27	12.57±0.15[a]	0.09	14.24±0.44[c]	0.25
	Carrageenan yield (%)	35±6.72[c]	3.88	28.43±2.14[ab]	1.24	26.36±5.56 [a]	3.21
	Viscosity (cP)	696.67±5.77[c]	3.33	550±66.14[b]	38.19	383.33±26.02	15.02
The best of G3	Dry seaweed moisture content (%)	11.22±0.20[ab]	0.12	10.06±0.94[a]	0.54	10.98±0.57[ab]	0.33
	Water content of carrageenan yield (%)	1.83±0.58[c]	0.33	7.14±1.01[b]	0.58	6.01±0.99[ab]	0.57
	Carrageenan yield (%)	45.87±3.59[c]	2.07	40.60±0.53[bc]	0.31	34±5.29 [ab]	3.05
	Viscosity (cP)	966.67±19.09[e]	11.02	901.67±44.81[d]	25.87	800±0[c]	0
G3 population	Dry seaweed moisture content (%)	11.59±0.42[ab]	0.24	12.51±2.26[b]	1.30	12.02±0.85[ab]	0.49
	Water content of carrageenan yield (%)	6.83±1.15[ab]	0.66	6.17±1.26[ab]	0.73	5.16±0.24[a]	0.14
	Carrageenan yield (%)	35.87±5.22[ab]	3.01	38.93±5.48[bc]	3.16	29.90±1.85[a]	1.07
	Viscosity (cP)	812.5±25 [c]	14.43	605±5[b]	2.89	533.33±28.87[a]	16.67

Note: The same color numbers followed by different superscript letters show significant differences at the 5% test level (Duncan's Multiple Range Test).

Table 1 shows three mass selections (G1, G2, G3) with selection intensity and 30 days of rearing time, in which each variety showed a different DGR value (Table 1). The average number of harvests for each variety (Kupang, Lampung, Tarakan) populations of *K. alvarezii* G1, G2, and G3 showed a higher increase in weight in the next generation compared to the previous generation (Figure 4).

One of the essential criteria for carrageenan yield raw materials is determined by water content. Table 2 shows the moisture content of dried seaweed raw materials and carrageenan yield water content resulting from the extraction of dried seaweed raw materials produced in the mass selection treatment. The value for the range of dry seaweed moisture content required in SNI 2690: 2015 (BSN, 2017) is a maximum of 30%. The water content value in this study met the value required by SNI except for the raw material G1 of the Lampung variety. The best value for the water content of the dry rule was 36.50%, and the water content in carrageenan yield was 33.74%, slightly exceeding the SNI prerequisite value for dry seaweed moisture content.

In the percentage value of carrageenan yield content (%), the highest value was obtained from the *K. alvarezii* Lampung variety (Table 2) due to the G3 maintenance selection for all samples tested in this study. Likewise, the viscosity value (cP) tested showed the highest value of the *K. alvarezii* of the Lampung variety due to G3 maintenance selection for all samples tested. The carrageenan yield value required by the industry is ≥20%. This study's carrageenan yield percentage value exceeds the industry requirements and is generally higher than the carrageenan yield percentage value reported by Simatupang *et al.* (2021). Seed quality of *K. alvarezii* includes total sugar content (Table 3), levels of the phytohormones IAA, Gibberellin, Zeatin, and Kinetin (Table 4), and carrageenan value (Table 2) accompanied by testing the thallus ash content and acid/silicate insoluble ash content (Table 3).

Table 3. Total Sugar Content, Ash Content, and Silicate Content thallus of *K. alvarezii*.

Treatment	Thallus of seed *K. alvarezii* variety					
	Lampung (n=3)		Tarakan (n=3)		Kupang (n=3)	
	Glucose Total (% w/w) P-value 0.000					
	Average	S.E	Average	S.E	Average	S.E
Non selected	2.37±0^{abc}	0	1.60±0^{a}	0	1.86±0^{a}	0
The best of G3	2.89±0.51^{bcd}	0.29	3.73±1.16^{d}	0.67	3.05±0.41^{cd}	0.24
G3 population	2.40±0.41^{abc}	0.24	2.45±0.03^{abc}	0.02	2.08±0.51^{ab}	0.29
	Ash Content					
The best of G3	8.27%		7.85%		4.67%	
G3 population	4.06%		7.18%		5.38%	
	Silicate Content					
The best of G3	0.77%		1.83%		0.27%	
G3 population	0.17%		1.16%		1.32%	

Note: The same color numbers followed by different superscript letters show significant differences at the 5% test level (Duncan's Multiple Range Test).

Table 4. Phytohormone Levels.

Treatment	Hormone P-value 0,022	Seed *K. alvarezii* variety					
		Lampung (n=3)		Tarakan (n=3)		Kupang (n=3)	
		Phytohormon levels (mg/L)	S.E	Phytohormon levels (mg/L)	S.E	Phytohormon levels (mg/L)	S.E
Non selected	IAA	18.35±0.37^{b}	0.21	19.81±0.58^{c}	0.33	18.02±0.18^{b}	0.10
	Gibberellin	26.64±0.47^{b}	0.27	25.24±1.92^{ab}	1.11	23.11±0.16^{a}	0.09
	Zeatin	16.93±0.06^{cd}	0.03	17.73±0.42^{d}	0.24	16.33±0.96^{c}	0.55
	Kinetin	9.46±0.69^{c}	0.40	7.60±0.12^{b}	0.07	7.45±0.22^{b}	0.13
The best of G3	IAA	23.19±1.66^{e}	0.96	21.54±0.69^{d}	0.40	28.97±0.17^{f}	0.10
	Gibberellin	33.48±0.41^{c}	0.24	39.65±0.01^{e}	0.01	35.31±0.24^{cd}	0.14
	Zeatin	14.33±0.26^{b}	0.15	18.97±0.47^{c}	0.27	16.36±1.15^{c}	0.66
	Kinetin	5.87±0.01^{a}	0.01	5.73±0.70^{a}	0.40	8.70±0.15^{c}	0.09
G3 population	IAA	16.17±0.06^{a}	0.03	21.77±0.83^{de}	0.48	21.24±1.36^{cd}	0.79
	Gibberellin	26.12±2.99^{b}	1.73	35.88±0.84^{d}	0.48	24.91±1.19^{ab}	0.69
	Zeatin	19.46±0.09^{c}	0.05	13.09±0.01^{a}	0.01	15.07±0.90^{b}	0.52
	Kinetin	5.71±0.32^{a}	0.18	5.73±0.63^{a}	0.36	5.90±0.61^{a}	0.35

Note: The same color numbers followed by different superscript letters show significant differences at the 5% test level (Duncan's Multiple Range Test).

Physiological diversity of *K. alvarezii* includes different varieties (Lampung, Kupang, Tarakan), different generations (G0, G1, G2, and G3), and different treatments between samples from the best families (S) and populations (P). The Levene test (Table 2, Table 3, and Table 4) show high diversity (sig <0.05) in the character of the water content of the thallus G2 and G3, the content of the yield of carrageenan G0 and G2, and the viscosity of the carrageenan G1, G2 and G3. Likewise, the results of testing the ash content and acid-insoluble ash content (total silicate content) were different for each variety. The highest Lampung G3 was the best at 8.27%, and the best Tarakan G3 was 1.23%.

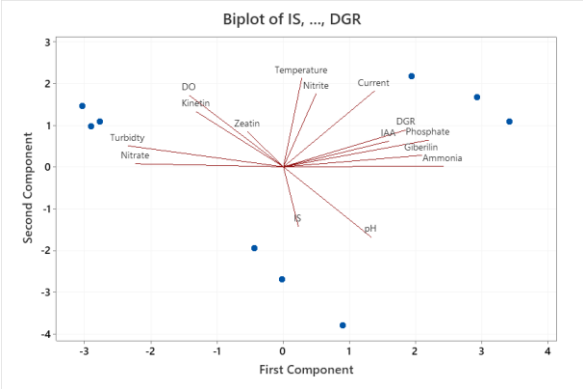


Figure 5. Biplot analysis of environmental factors and quality of *K. alvarezii* seaweed cultivated outdoor at Amal Beach

Biplot analysis is used to see the relationship between the variables. It shows that the vector

variable DGR coincides with the vector variables IAA, Phosphate, Gibberellin, and Ammonia. This shows that the four variables are correlated. In a biplot, the narrower the angle between the two vector variables, the stronger and more positive correlation variable. Furthermore, it is followed by a regression analysis (Figure 5) to see whether there is influence from the correlated variables, with the response variable used being the DGR variable.

Regression analysis was performed using stepwise regression analysis. Based on the results above, it can be seen that the p-value of ammonia is $0.036 < \alpha = 0.05$. This shows that ammonia affects DGR. The relationship between Ammonia and DGR can be seen from the regression equation: DGR=3.732+4.89 ammonia. From the regression equation, it is obtained that the Ammonia coefficient is 4.89, which means that for every increase in the Ammonia value by one unit, the estimated mean value of DGR will increase by 4.89.

Discussion

The DGR values (Table 1) of the three selected varieties cultivated outdoors ranged from from 3.62-5.90%, which were still categorized as good. This is in line with Fadilah *et al.* (2016), who state that the DGR of other seaweed is categorized as good if the average DGR value is at least 3%/day. It is also in line with Arjuni et *al.* (2018), who stated that the weight of seaweed is considered profitable if the DGR value is above 3 %/day. Unlike the case with the selection of fish or shrimp, the selection is carried out through mating the parents to get the next generation. In selecting seaweed varieties, seed production in each generation is only carried out through a vegetative propagation process in obtaining the next generation, known as the seed production cycle (Pongmasak *et al.,* 2011). Thus, the selection response can only be estimated based on the increase in the DGR of selection for each generation. Internal control in this study was obtained from *K. alvarezii* of the Lampung variety with the criterion of the medium weight of 60% of the population. A selection was made on the clumps with the highest 20% weight to become the next generation of seeds. The lowest 20% weight was cut off.

The high DGR value of internal control 1 came from *K. alvarezii* var.Lampung with the intermediate weight category (60%) of the Lampung G1 population was re-cultivated during the G2 cycle as an internal control. The Lampung variety comes from the F60 tissue culture, which is suspected of having more regenerative cell growth through embryogenic callus development and morphogenesis (Sulistiani *et al.,* 2014). In line with this, *K. alvarezii* obtained the seeds with the highest DGR value (5.90%) from *K. alvarezii* var.Lampung G3, whose seeds came from the F60 development of the BBPBL Lampung' nursery, has a clear track record of F1 parent obtained from tissue culture propagation at SEAMEO-Biotrop, Bogor. At the end of the mass selection rearing cycle in Amal Coastal Waters, the DGR G3 value of *K. alvarezii* var.Lampung, Kupang, and Tarakan experienced an increase in DGR compared to the DGR values of internal control 1 and internal control 2, which experienced a decrease in DGR (Table 1). The DGR value of the controls in this study was still higher than the DGR value reported by Rukisah et al. (2020), which decreased from the first week of DGR 3.56%/day to 1.58%/day in the third week of *K. alvarezii* cultivation at Amal Beach.

The decrease in DGR occurred in G2 Kupang due to oyster attacks at the research location (Amal Coastal Waters) in April and May 2021, which inhibited seaweed growth (Wt) *K. alvarezii* var. Kupang compared to other varieties. Tarakan variety is relatively resistant to oyster spat attacks compared to var. Kupang because of var.Tarakan has already been adaptive to the Amal Coastal Waters and is a local variety. Likewise, *K. alvarezii* var.Lampung seedlings *were* more resistant to oyster spat than var.Kupang because of var.Lampung was thought to have optimum cell strength and was also adaptive to local water salinity (30 ppt) compared to the original salinity of *K. alvarezii* var.Kupang. Kupang's salinity value of the waters can reach 34-36 ppt (Simatupang *et al.,* 2021). In addition, the size of the thalus of *K. alvarezii* var.Lampung was smaller in diameter and different from the thallus of *K. alvarezii* var.Kupang. In the early period, the thalus of *K. alvarezii* var.Kupang has a thicker and larger diameter than the thallus of var. Tarakan and var.Lampung so that the oyster spat sticks more efficiently and causes the thallus to develop a secondary infection in the form of stem rot disease. This was thought to trigger the decrease in the DGR value of G2 *K. alvarezii* var. Kupang, conditions improved again as oyster spat decreased at Amal Coastal Waters in the following month. This is very different from what Oedjoe et al. (2022) reported: this *K. alvarezii* variety can grow in NTT waters until it reaches a DGR value of 5.19-6.25%/day in the first week of cultivation.

The carrageenan yield ranged from 23.16-45.87%. It showed higher G3 than G2 and G1; the Lampung variety *K. alvarezii* was higher than the Kupang and

Tarakan varieties. The Kupang variety from selection was lower than the thallus sample *K. alvarezii var.*Kupang without selection. It was suspected that the age of the seaweed thallus without selection was older than that of the selected thallus samples maintained in this study. The maintenance length of thallus samples followed the mass selection protocol for 30 days (Pongmasak *et al.,* 2011). This is in line with the results of Junaidi *et al.* (2018), who stated that the harvesting age *of K. alvarezii* affected its carrageenan content. The addition of the harvesting age *K. alvarezii* will increase its carrageenan content. Research by Widyastuti (2010) states that *K. alvarezii* harvested at 15 days of age has a carrageenan content of around 40% and increases to around 50% at 45 days. In addition, the carrageenan content is strongly influenced by the location where it grows. Carrageenan content in seaweed ranges from 54-73% (Periyasamy *et al.,* 2014; Khambhaty *et al.,* 2012; Wenno *et al.,* 2012).

This lowest viscosity value of carrageenan yield in this study was 383.33 ± 26.02 cP obtained from thallus G2 sample population var.Kupang, while the highest 901.67 ± 44.81 cP was obtained from the best thallus sample, *K. alvarezii* G3 var.Lampung. The viscosity value in this study was high, as reported in Ohno *et al.* (1994); the viscosity values of carrageenan yield *K. alvarezii* cultivated in subtropical locations ranged from 580-1240 cP with a percentage of carrageenan yield of 27.6-43.5%. The viscosity of hydrocolloids is influenced by several factors, including carrageenan concentration, temperature, type of carrageenan, molecular weight, and the presence of other molecules (Towle, 1973; FAO, 1990). If the carrageenan concentration increases, the viscosity increases logarithmically (Diharmi, 2016). The carrageenan viscosity value required by FAO (2004) is > 5 cP, and the viscosity value in this study still meets these criteria.

The moisture content of dry thallus and carrageenan yield also supported the carrageenan yield in this study. The moisture content of raw material is essential to maintain the material's quality, especially in long-term storage. In addition, drying shrinkage affects the selling price. Dry seaweed preferred by the industry ranges from 37% and below because it will not get damp during the raw material distribution chain process or be damaged (mouldy) when stored for a long time. For the value of carrageenan water content, FAO (2004) and SNI 8391-1:2017 (BSN, 2017) require a maximum value of 12%. The yield of G3 carrageenan meets these criteria. This is presumably because the other samples in this study still contained relatively high water content in the drying process of dried seaweed thallus raw materials G1 and G2, which were then extracted to yield carrageenan.

Measurement of the total sugar content of seaweed seedlings (30 days) in this study was used to estimate the potential levels of seaweed carrageenan at harvest age (45 days) (Fadilah *et al.,* 2016). Of the three varieties tested and each based on the criteria without selection, G3 the best/selected sample weight and G3 population (Table 3), the total sugar content value of the best/selected G3 sample has the highest percentage of total sugar content compared to other criteria, with the highest value of total sugar content of *K.* alvarezii var.Tarakan was 3.73 ± 1.16%. This provides valuable information that the candidate *K. alvarezii* var.Tarakan has opportunities for developing seedlings (nurseries) that apply the mass selection protocol. By using *K. alvarezii*, the local local variety K. alvarezii, *var.*Tarakan, which is thought to have the potential to produce optimal carrageenan levels at harvest age (45 days), is more profitable because the availability of local seeds (*K. alvarezii* var.Tarakan) is more adaptive to the location. Compared to other varieties, Amal Beach waters must be imported first (via air transportation) to Tarakan City.

The harvesting age *of K. alvarezii* affects the ash content of carrageenan (Wenno *et al.*, 2012). Furthermore, it was stated that the highest ash content was found in *K. alvarezii*, harvested at 55 days of age, and the lowest at 40 days of harvest. Increasing harvest time tends to cause the ash content of carrageenan flour to increase (Junaidi *et al.,* 2018). In this study, the ash content (dry basis) and acid/silicate insoluble ash content (in 1% H_2SO_4) ranged from 4.06-8.27% and 0.27-1.83%, and still met the criteria for carrageenan quality standards recommended by FAO (2004) and SNI 8391-1: 2017 (BSN, 2017). Both require a maximum carrageenan value of 40% for ash content and <2% for acid/silicate insoluble ash content (in 1% H_2SO_4). However, the sample used in testing the ash content in this study was still in the form of dry thallus aged 30 days (not in the form of carrageenan samples).

Phytohormones are organic compounds made by plant cells and are generally translocated to other parts, are active even in microscopic levels (10^{-5}-10^{-6} mm), and cause-specific biochemical, physiological, and morphological changes responses. Plants generally have a high degree of developmental plasticity and display various types of tissue or organ regeneration (Mastuti, 2017). This regenerative capacity can be increased by hormones and nutrients supplied by the environment. Phytohormones are

factors that greatly determine the success of the growth and differentiation of plant cells (Mastuti, 2017).

Seaweed seeds *K. alvarezii* had higher levels of the phytohormones IAA, gibberellin, and zeatin than the thallus *K. alvarezii* G3 population and without selection. However, the analysis of the phytohormone kinetin for the *K. alvarezii* thallus sample without selection showed a higher value than the G3 thallus for the population and the best sample. In contrast, the results of Fadilah *et al.* (2016) reported that selected *K. alvarezii* had a 15.52% greater concentration of the hormone kinetin than the internal control. This difference is due to the internal control sample criteria in the study by Fadilah *et al.* (2016), which is thought to be the same age as *K. alvarezii* (30 days). Meanwhile, in this study, samples of *K. alvarezii* thallus without selection were obtained during the initial arrival of seedlings transported by air from Lampung and Kupang to Tarakan City with different thallus ages, which exceeded 30 days of *K. alvarezii*.

The results of the phytohormone test in this study obtained the highest concentration of gibberellin 23.11-39.65 mg/L, then IAA 16.17-68.97 mg/L, zeatin 13.09-18.97 mg/L and the lowest levels of phytohormone kinetin 5.71-9.46 mg/L which affects various physiological processes of seaweed seedlings *K. alvarezii*. The auxin hormone can influence many physiological changes in cells through its ability to increase the permeability of the plasma membrane to organic materials so that the absorption of organic matter into cells becomes higher (Taiz & Zeiger, 2002). All cytokinins have a chemical structure similar to zeatin and kinetin - the interaction of auxin and cytokinin results in morphogenesis. Cytokinins suppress thallus growth by decreasing cell size and reducing cell division.

Gibberellin, as a plant hormone, plays a role at the cellular level by influencing some physiological processes that cannot be explained clearly (Lakitan 2008). In some cases, gibberellin enhances RNA and protein synthesis. Gibberellin stimulates DNA synthesis for cell expansion. Gibberellin also promotes the conversion of tryptophan into auxin and inhibits the operation of auxin oxidase so that auxin levels become higher (Taiz and Zeiger 2002). According to Jennings (1970), *in vitro* seaweed, *Gracilaria verrucosa* has a higher growth rate than the control with adding gibberellin at a concentration of 0.02-0.50 mg/L. In influencing growth, gibberellin does not work alone but optimally with auxins and cytokinins (Mulyaningrum *et al.*, 2013).

Physiological characteristics of the thallus of *K. alvarezii* seedlings result from selection G3 var.Lampung (origin of tissue culture) produces a higher value than other varieties (non-tissue culture). Kasim *et al.* (2021) stated that the average growth of *K. alvarezii* was higher because they had more branches than local seeds. Thallus, which has many branches, can maximize the absorption of nutrients from the waters. Carrageenan and biomass are closely related to the optimum harvest time. The optimum harvest time for *Kappaphycus* is 8-9 weeks (Villanueva *et al.*, 2011). Generally, seaweed with high DGR has higher carrageenan (Naguit *et al.*, 2009). This is in line with research by Pongarrang *et al.* (2013), who stated that seaweed growth correlated with carrageenan content; that is, when growth is high, carrageenan content is also high.

The biplot analysis (Figure 5) shows that the DGR variable vector coincides with the IAA, Phosphate, Gibberellin, and Ammonia vectors. Ammonia stepwise regression analysis (sig<α) affects DGR with an Ammonia coefficient value of 4.89; that is, for each increase in the Ammonia value by one unit, the estimated mean value of DGR will increase by 4.89. Algae can generally assimilate various nitrogen sources, but Ammonium is preferred when available in waters, and algae only make full use of Ammonium (Chai *et al.*, 2021). The availability of nitrogen (N_2) can be measured in bioavailable forms, such as oxidized nitrogen (nitrates and nitrites) and ammonia, and as total nitrogen, which includes organic and bound forms of nitrogen. Several species of algae, such as *Chlorella* sp., are tolerant of organic pollution, but at high concentrations, ammonia inhibits algae's growth and physiological activity (Siong *et al.*, 2021). The mechanism of ammonia's effect on algae can be viewed from three aspects: growth characteristics, pigments and photosynthetic performance, and biochemical composition. The concentration at which ammonia toxicity affects algal growth varies considerably under mixotrophic and autotrophic conditions (Tam *et al.*, 1996). In general, environmental conditions of water quality in Amal Beach, Tarakan City, is still suitable for supporting the growth and cultivation of *K. alvarezii*.

Conclusion

Seed of seaweed *K. alvarezii*, a result of mass selection cultivated outdoors at Amal Beach for three mass selections, showed the best increase in G3 daily growth rate ranging from 4.84-5.90%/day with carrageenan yield of 34.00-45.87%. DGR positively correlates with the hormone components IAA, Gibberellin, Ammonia, Phosphate, and pH.

Seaweed *K. alvarezii* var.Lampung seed is the best candidate to be developed at the seaweed cultivation site in Tarakan City through mass selection.

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