Growth and adaptability of *Kappaphycus alvarezii* from different genetic sources in an indoor cultivation system

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Abstract. The potential for seaweed aquaculture in Tarakan, North Kalimantan, can be managed appropriately by applying selection rules for *Kappaphycus alvarezii* seeds and measurable, targeted and adaptive cultivation technology, in order to increase productivity and sustainability. Currently, the main problem faced by *K. alvarezii* cultivators is the low quality of the seeds from the cultivation. This research investigated the growth of *K. alvarezii* from different genetic sources, cultivated in a controlled system, under the influence of water quality and algae physiology. The design used in this study was a completely randomized design (CRD). *K. alvarezii* varieties with different genetic sources from Lampung (F60 tissue culture), Kupang (non-tissue culture) and Tarakan (non-tissue culture) were cultivated for 51 days in an indoor cultivation system. The physiological characteristics' variance of indoor cultivated seeds was analysed at a 5% significance level, using SPSS version 22 software. The principal component analysis (PCA) identified the most significant parameters, using the Minitab 19 program. In indoor seedlings of the *K. alvarezii* varieties Lampung, Kupang and Tarakan, the daily growth rate (DGR) values were 0.013±0.35 % day^{-1}, -1.103±0.62 % day^{-1} and 0.707±0.23% day^{-1}, respectively. The multivariate main component analysis (PCA) showed that the first combination (DO, water temperature, salinity, Indole-3-Acetic Acid, gibberellins, zeatin, kinetin, ammonia, nitrite, nitrate, phosphate, weight) described 29% of the whole data structure. From the regression equation, the Indole-3-Acetic Acid (IAA) coefficient value was 374.2, which means that for every increase in the IAA value by one unit, the estimated mean weight value will increase by 374.2 units. At the same time, IAA is a type of auxin that induces elongation growth and apical dominance.

Key Words: algae, growth rate, phytohormone, seaweed, thallus, water quality.

Introduction. The potential for seaweed aquaculture in Tarakan, North Kalimantan, can be managed appropriately by applying selection rules for seeds *Kappaphycus alvarezii*, and measurable, targeted and adaptive cultivation technology, in order to increase productivity and sustainability. Currently, the main problem faced by seaweed cultivators *K. alvarezii* is the low quality of the seeds from the cultivation. The decrease in environmental carrying capacity due to global warming also impacts the vulnerability of *K. alvarezii* to pests and diseases (Largo et al 2017), which are detrimental to the seaweed cultivation business in Tarakan.

The selection of varieties is one method to increase the growth rate of seaweed. This research-based on daily growth rate (DGR) parameters and the selection method referred to the seaweed selection protocol *K. alvarezii* that had been developed (LP2BRL 2013). The results of previous studies by Pong-Masak et al (2014) reported that the application of the varietal selection protocol on seaweed *K. alvarezii* increased its growth by 15%-42%. The high growth of the selected seaweed is also thought to be due to the different distribution of plant growth hormones among the clumps or branches of the seaweed. Fadillah et al (2016) reported that the selected *K. alvarezii* seaweed, in addition to having a higher growth, also had a high concentration of plant growth hormones, especially kinetin, which was by 15.52% greater than other non-selected seaweeds (internal control).

Most cultivated *K. alvarezii* seeds propagation occurs asexually, by thallus fragmentation (Roleda et al 2021). The identification based on the morphology (phenotype) is relative; the level of diversity depends on the environmental factors which also determine the genetic potential. *K. alvarezii* seeds in Tarakan come from different sources but there is no track record of the specific genetic sources, at the moment.

The mass selection method was successfully applied to obtain seedlings of *K. alvarezii* quality (Pong-Masak et al 2013). Efforts to provide superior broodstock for seaweed nurseries urgently require adaptation techniques to maintain the survival rate of seaweed, consisting of tissue culture plantlets or seeds *K. alvarezii* to be developed (Budiyanto et al 2019). Acclimatization is a technique of adjusting seedlings from the culture stage to the marine environment. The acclimatization of cultured seaweed seedlings can be carried out in the laboratory, using an aquarium, in an indoor container, using a concrete tub with a more extensive maintenance system, and finally in an outdoor system (aquaculture in the sea) using a net (Karamba Jaring Apung) adapted to the size of the thallus seeds. When *K. alvarezii* reaches its optimum level, it can be tied to a rope for further development in the nursery. The challenge in adapting seaweed seeds is the attack of the white disease, due to the environmental change. Environmental factors that trigger seaweed thallus stress at various stages of acclimatization are nutrient suitability, salinity levels, temperature, oxygen and light (Parakkasi et al 2020).

Mass selection activities in land farming systems already have standards and benchmarks, useful in roguing. Roguing in agriculture identifies and eliminates stray plants (Murphy et al 2005). The purpose of roguing is to maintain purity and genetic quality. The characterization of seaweed from different genetic sources can identify the type of intersection, through a controlled cultivation approach in indoor systems, due to the technical constraints in the field during aquaculture in the sea (in nature), such as the preparation for planting seaweed seedlings. The acclimatization treatment (pre-cultivation) requires a physiological and adaptation approach in a controlled tank, that aims at minimizing the growth-inhibiting factors of *K. alvarezii* and at evaluating the seedling characteristics in a new environment. Environmental factors influence seaweed growth; for example, several research reports on the carrageenan content as depending on water quality parameters like temperature, light intensity and nutrients. Nitrate and phosphate play a vital role in algae growth, including seaweed, while nitrogen is the main limiting factor as nutrient for algae growth in marine ecosystems. The success of seaweed cultivation requires knowledge on its adaptation, physiology and environmental factors (Azanza & Ask 2017). Martins et al (2011) reported that nutrient deficiency in some species of algae could cause growth decline. This research investigates the growth of *K. alvarezii* from different genetic sources, cultivated in a controlled system, in order to obtain a practical approach to seaweed growth, related to several water quality parameters and to the algae physiology, that significantly affect the development of *K. alvarezii*.

Material and Method

Description of the study. *K. alvarezii* was cultivated in concrete tanks of algae culture at the local shrimp hatchery (Balai Benih Udang, BBU), Amal Beach from February 28, 2021, until April 21, 2021. The sampling of water quality data (salinity, temperature, pH, and DO) was carried out in situ. The sampling of chemical water parameters, i.e., ammonia, nitrate, nitrite and phosphate, was carried out at the Water Quality Laboratory of FPIK-UBT Tarakan. The carrageenan yield analysis at the Nutrition and Disease Laboratory of FPIK UBT and the phytohormone measure at the Bogor Agrochemical Residue Laboratory.

The design used in this study was a completely randomized design (CRD). The treatment used *K. alvarezii* varieties with different genetic sources, from Lampung (F60 tissue culture), Kupang (non-tissue culture) and Tarakan (non-tissue culture), cultivated for 51 days in an indoor cultivation system, using concrete tanks with a capacity of 10 tons (Figure 1) and a density of 95 g m^{-3} (Marisca 2013; Sulistiani & Yani 2014). The treatments of different seeds in each indoor system were repeated three times. Clumps grouped seaweed seeds (by 38), with a weight of ±25 g for each clump tied to a loop on a rope (with spaces of 15 cm between the loops).

The indoor cultivation system (Figure 1) used in this study was a closed system with controlled aeration and 100% water change carried out every week or sixth day of maintenance (except for the first two weeks and the final two weeks of the study, when there were no water changes). The total weight of the seaweed harvest was measured at the beginning and end of maintenance. *K. alvarezii* requires current movements (≥0.1 m s^{-1}) and oxygen to carry out respiration. So, in each maintenance concrete tank, four tight aerators are placed (connected to an aeration hose along ½ of the total water column of each tank, using 3 PL100 blowers for the 9 bath replicates), producing water movement and circulation of O_2. The dissolved oxygen (DO) concentration suitable for the seaweed is 5 mg L^{-1}, depending on air diffusion, water exchange, photosynthetic results and aeration processes.

Figure 1. Indoor tub for *Kappaphycus alvarezii* seedling cultivation (original photo).

Observations. The initial and final weight of *K. alvarezii* seeds harvest were measured. The DGR is calculated using the initial weight value (W0) and the biomass obtained over a specific time (Wt), using the formula DGR = ((ln Wt – ln Wo)/t) x 100). The growth regulators of phytohormones from *K. alvarezii* in the form of IAA, gibberellin, zeatin and kinetin preparation were used in Linskens & Jackson (1987), followed by measurements using HPLC. The carrageenan analysis (AOAC 1995) include the yield content, weight loss, moisture content, percent loss, thallus moisture content, thallus ash content and silicate (acid insoluble ash content).

Statistical analysis. Parameters tested statistically were seaweed daily growth rate (DGR), final weight (harvest), and phytohormones levels. Harvest data, hormones, and carrageenan were analyzed for their variance at a 5% significance level, using the SPSS® version 22 software. The principal component analysis (PCA) test was conducted to determine the significant parameters in this study using MINITAB® software 19 program.

Results. Physiological characters also support the morphological characters in this study (Table 1). Physiological characteristics include phytohormones (IAA, Giberilin, Zeatin, and Kinetin) and carrageenan extraction from *K. alvarezii* seeds (with different genetic sources, from Lampung, Kupang and Tarakan). The carrageenan test analyzes weight loss data, moisture content of dry thallus, yield, viscosity, ash content and acid-insoluble ash content (total silicate content).

Table 1

Physiological characteristic of indoor cultivated *Kappaphycus alvarezii* seeds

Variable	*Unit*	*Genetic source K. alvarezii*		
		Lampung	*Kupang*	*Tarakan*
Hormone physiology and harvest				
IAA	mg L^{-1}	19.977±2.968^a	16.389±0.710^a	19.490±0.946^a
Giberilin	mg L^{-1}	26.642±0.666^a	17.126±1.538^b	19.760±2.112^b
Zeatin	mg L^{-1}	21.132±1.241^a	17.080±1.223^b	18.669±1.142^b
Kinetin	mg L^{-1}	10.514±0.520^a	7.803±0.409^b	7.952±1.081^b
Total weight harvested	g	965.67±166.51^b	560.33±186.87^c	1369.67±167.43^a
Daily growth rate (DGR)	% day^{-1}	0.013±0.35^a	-1.103±0.62^b	0.707±0.23^a
Carrageenan				
Weight loss	%	90.60±0.34^a	89.57±0.49^a	89.63±0.64^a
Moisture content of dry thallus	%	19.33±4.16^a	12±3.46^a	16.67±3.06^a
Carrageenan	%	42.77±7.08^a	22.63±1.88^b	26.83±3.90^b
Viscosity	centipoise	305±5^a	208.33±2.89^b	186.67±2.89^c

The numbers in the same row followed by different superscript letters show significant differences at the 5% test level (Duncan's Multiple Distance Test).

The physiological diversity of *K. alvarezii* had a wide variety of hormone levels, total weight of harvest and daily growth rate (DGR), depending on its genetic sources, as shown by the Levene test (Table 1), with sig<0.05. A high diversity was recorded for the yield of carrageenan and viscosity value. The ash content test resulted in different values for each variety, namely Lampung, with 4.93%, Kupang, with 4.88% and Tarakan, with 3.22%. Likewise, the test results for acid-insoluble ash content (total silicate content) differed for each variety, namely 0.75% for Lampung, 0.68% for Kupang and 0.69% for Tarakan. Results shown in Table 2 present the water samples quality during the maintenance in indoor containers.

The principal component analysis (PCA) is used to overcome collinearity problems in multiple linear regression, through the eigenvalue approach (Lv & Gu 2012). A researcher can identify the significant parameters with a minimum loss of information (Singh et al 2004). This study's PCA used the Minitab version 19 program to determine the eigenvalues (Table 3), the scree plot (Figure 2), the eigenvectors (Table 4), the biplot analysis (Figure 3), and the regression analysis (Figure 4).

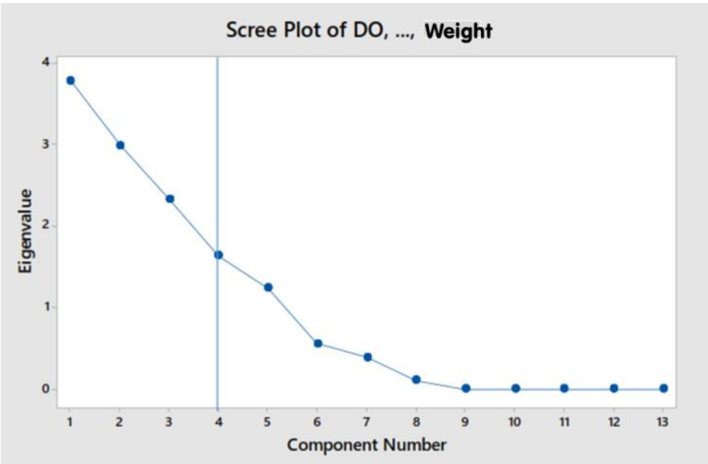

Figure 2. Screen plot for *Kappaphycus alvarezii* indoor cultivation.

Four components (Figure 2) were identified, based on the above results (Eigen analysis and scree plot), explaining 82.4% of the variability. The X-axis of the scree plot shows the eigenvalues of each component, while the Y-axis shows the type of PC (PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8, PC9, PC10, PC11, PC12, and PC13).

Table 2

The value of water quality which is the average value during 51 days of cultivating *Kappaphycus alvarezii*

Parameter	Standard	Code of completely random design								
		Variety Kupang (K)			Variety Lampung (L)			Variety Tarakan (T)		
		KD	KT	KB	LT	LD2	LD1	TT1	TT2	TD
Temperature	26-32 (SNI 2010)	27.97	27.93	27.64	27.87	27.75	27.82	27.99	28.03	27.87
Salinity (‰)	29-34 (SNI 2010)	30	30	30	30	30	30	30	30	30
DO (mg L^{-1})	≥5 (SNI 2010)	8.02	7.99	8.02	8.03	8.06	7.96	7.96	7.98	8.01
pH	6.8-8.8 (SNI 2010)	8.04	8.03	8.04	8.03	8.05	8.07	8.04	8.05	8.05
Ammonia (mg L^{-1})	≤0.300 (KepMen LH 51/2004)	0.052	0.056	0.060	0.060	0.057	0.045	0.052	0.041	0.041
Phosphate (mg L^{-1})	0.020-1.0 (Lakitan 2000)	0.050	0.062	0.045	0.057	0.061	0.052	0.050	0.060	0.057
Nitrate (mg L^{-1})	0.040-0.100 (Effendi 2003)	0.170	0.233	0.176	0.200	0.180	0.221	0.205	0.215	0.166
Nitrite (mg L^{-1})	≤0.060 (CCRM 1987)	0.020	0.017	0.020	0.018	0.017	0.019	0.019	0.019	0.018

Table 3

Eigen analysis of the correlation matrix for the *Kappaphycus alvarezii* indoor cultivation

Parameter	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*	*PC7*	*PC8*	*PC9*	*PC10*
Eigenvalue	3.7717*	2.9820*	2.3274*	1.6333*	1.2368	0.5554	0.3848	0.1085	0.0000	0.0000
Proportion	0.290*	0.229*	0.179*	0.126*	0.095	0.043	0.030	0.008	0.000	0.000
Cumulative	0.290*	0.520*	0.699*	0.824*	0.919	0.962	0.992	1.000	1.000	1.000

* shows the largest contribution value of each variable in the component.

Table 4

Eigenvectors of *Kappaphycus alvarezii* cultivation indoor

Variable	*PC1*	*PC2*	*PC3*	*PC4*
DO	0.286	0.236	-0.171	-0.514**
Water temperature (TA)	-0.358**	-0.105	0.381**	-0.076
pH	-0.181	0.174	-0.364**	0.408**
Salinity	0.050	0.166	0.004	0.309
IAA	-0.461**	0.061	0.037	-0.197
Giberilin	-0.198	0.455*	-0.218	0.066
Zeatin	-0.439**	0.052	-0.237**	-0.278
Kinetin	0.105	0.406*	-0.270	0.306**
Ammonia	0.255	0.308	0.244	-0.151
Nitrate	0.175	-0.011	-0.471**	-0.438**
Nitrite	0.074	0.423*	0.310	-0.117
Phosphate	0.055	-0.448*	-0.362	0.051
Weight (bobot)*	-0.443**	0.152	-0.078	-0.151

* and ** indicate the largest contribution value of each variable in the component.

For each eigenvector, the coefficient value of each variable was presented in Table 4. Based on the results above, the equations for each PC are as follows:

1. PC1 = 0.286 DO – 0.358 TA (water temperature) - 0.181 pH + 0.050 Salinity – 0.461 IAA – 0.198 gibberellin – 0.439 zeatin + 0.105 kinetin + 0.255 ammonia + 0.074 nitrite + 0.175 nitrate + 0.055 phosphate – 0.443 weight (bobot);
2. PC2 = 0.236 DO - 0.105 TA (water temperature) - 0.174 pH + 0.166 salinity – 0.061 IAA + 0.455 gibberellin + 0.052 zeatin + 0.406 Kinetin + 0.308 Ammonia + 0.423 nitrite - 0.011 nitrate – 0.448 phosphate + 0.152 weight (bobot);
3. PC3 = -0.171 DO + 0.381 TA (water temperature) – 0.364 pH + 0.004 salinity + 0.037 IAA - 0.218 gibberellin – 0.237 zeatin – 0.270 kinetin + 0.244 ammonia + 0.310 nitrite - 0.471 nitrate – 0.362 phosphate – 0.078 weight (bobot);
4. PC4 = -0.514 DO - 0.076 TA (water temperature) + 0.408 pH + 0.309 salinity – 0.197 IAA + 0.066 gibberellin - 0.278 zeatin + 0.306 kinetin - 0.151 ammonia – 117 nitrite – 0.438 nitrate - 0.117 phosphate - 0.151 weight (bobot).

The absolute value of the weight's contribution coefficient in PC1 is higher than the total weight's contribution in other PCs. Furthermore, IAA, zeatin and water temperature (TA) also have a considerable contribution to PC1, indicating a positive correlation with the weight.

The biplot analysis is used to see the relationship between the variables. Based on the results of the biplot, it can be seen that the weight variable's vector coincides with the vectors of the variables IAA, zeatin and TA (water temperature). Biplot analysis shows that the four variables are correlated. The narrower the angle between the two vector variables in a biplot, the higher the positive correlation. Furthermore, the regression analysis (Figure 4) shows whether the correlated variables have an effect.

The analysis of variance (ANOVA) presents the statistical and probability values of the F test. The model summary table presents the coefficient of determination. Regression equation contains independent variable X (IAA), dependent variable Y (weight), constant 374.2, P-value=0.002<0.05 (significant).

A stepwise regression analysis was performed with a p-value of IAA of 0.002<=0.05, showing that IAA affects weight, according to the regression equation: weight=-6043+374.2 IAA, which means that for every increase in the IAA value by one unit, the estimated mean weight value will increase by 374.2 (Figure 4).

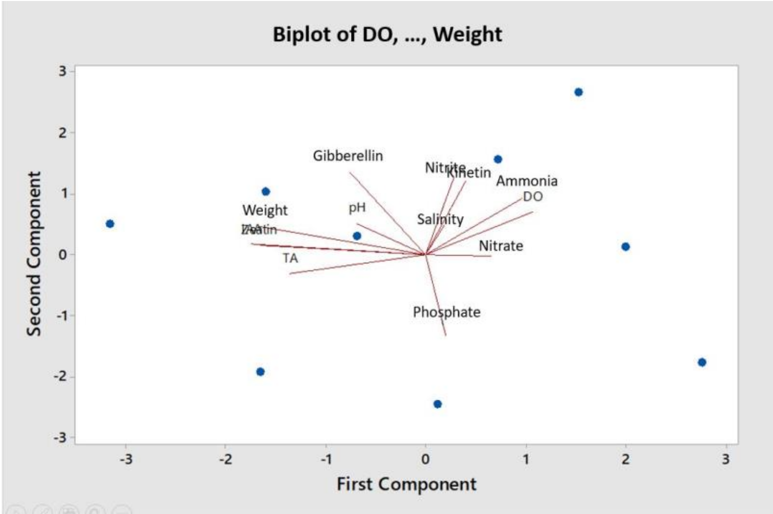

Figure 3. Biplot analysis of indoor *Kappaphycus alvarezii* cultivation.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	905405	905405	24.52	0.002
IAA	1	905405	905405	24.52	0.002
Error	7	258489	36927		
Total	8	1163894			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
192.164	77.79%	74.62%	47.01%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-6043	1417	-4.27	0.004	
IAA	374.2	75.6	4.95	0.002	1.00

Regression Equation

Weight = -6043 + 374.2 IAA

Figure 4. Regression analysis with response variable weight: DF-Degree of freedom; Adj SS-Adjusted sum square; Adj MS-Adjusted mean square; S-Standard error; R-sq-R square.

Discussion. Daily Growth Rate (DGR % day^{-1}) is one of the physiological characteristics used as a benchmark for seaweed growth in aquaculture. Seaweed cultivation activities are categorized as good if the average DGR is at least 3% (Pong-Masak et al 2015; Fadilah et al 2016). The weakness in this study was the high mortality rate (caused by a white disease called ice-ice), with the lowest DGR value in the Kupang variety (Table 1), -1.103±0.62% day^{-1}, and a highest DGR in the Tarakan variety, 0.707±0.23% day^{-1}. *K. alvarezii* seedlings are generally low when reared in controlled containers, as in the study of Zuldin et al (2015), the highest DGR for the seed of *K. alvarezii* of the green flower variety was 1.460±0.06% day^{-1} and the lowest was 0.410±0.33% day^{-1}. A negative DGR value indicates growth inhibition resulting in a negative potential for decreasing total final thallus weight and even seedling death. Even negative DGR's were observed in the indoor cultivation of *K. alvarezii* of the Brown Tambalang variety (density of 2.4 g L^{-1}). The low value of indoor DGR in this study, similarly to that of Sulistiani et al (2012) and Zuldin et al (2015), is due to an ice-ice disease which attacked the thallus causing loss, rot and even death of *K. alvarezii*.

Orbita's (2013) research conducted in Barangay Doña Consuelo, Ozamiz City, showed that nitrate and salinity concentrations significantly affect seaweed in aquatic environments and have a dominant influence on the carrageenan yield of *K. alvarezii.* Seaweed culture *K. striatus* in the laboratory cannot survive at salinities below 24‰ or above 45‰ (Glenn et al 1990). Orbita (2013) also reported that temperature and salinity were the main environmental factors affecting the growth of *K. alvarezii* in the Philippines, where *K. alvarezii* recorded its maximum increase in June-September, with temperatures of 27.67°C-30.72°C and a salinity of 23.60–29.80‰. In this research (Table 2), the daily average values of physical and chemical water quality parameters, during the 51 days of cultivating in a semi-indoor system, were still suitable for supporting the growth of *K. alvarezii* seedlings.

The thallus attacked by the ice-ice disease will lose its pigment, so that it becomes white, rotten and falls off (the morphology of the tissue affected by ice-ice is a secondary result). Doty (1987) stated that ice-ice is a seasonal condition associated with environmental changes. According to Neish (2005), there is a high population of bacteria in the thallus tissue, which is attacked by an ice-ice disease, a secondary cause. Largo et al (1995) said that certain bacteria attack the seaweed thallus under stress (from abiotic factors). That can trigger a disease outbreak, such as the incident in the Philippines, where ice-ice appeared due to reduced sunlight intensity, salinity below 28‰, and high daily temperature amplitude. The ice-ice disease first appeared in this study because the weather conditions in the environment around the indoor maintenance tank were unstable; the air became suddenly frigid, due to heavy rain during the previous night, then it turned fast to hot air in the morning and evening; simultaneously, a power outage occured. The absence of a blower and aerator triggered stress on the thallus of the seaweed *K. alvarezii* of Kupang and Lampung varieties, and also on a smaller number of Tarakan varieties, resulting in ice-ice disease.

Hormones influence the growth and development of plants, as growth regulators. Compared to animals, plants generally have a high degree of developmental plasticity and display various tissue or organ regeneration capacities (Leliaert et al 2014). This regenerative capacity can be increased by hormones and nutrients supplied by the environment. Phytohormones are factors that determine the success of plant cell growth and differentiation. Phytohormones are synthesized in certain parts of the plant and translocated to other regions, causing biochemical, physiological, and morphological responses. Among the five known plant hormones (auxins, cytokinins, gibberellins, ABA, and ethylene), auxins and cytokinins stimulate cell division and control cell differentiation and morphogenesis (Mastuti 2017). According to Jennings (1970), in vitro seaweed, *Gracilaria verrucosa*, has a higher growth rate than control when gibberellins reach a 0.02-0.50 mg L^{-1} concentration. In influencing growth, gibberellins work optimally together with auxins and cytokinins (Mulyaningrum et al 2013).

Seeds of *K. alvarezii* of the Lampung variety had higher phytohormones than the Tarakan variety and the lowest was Kupang. The content of phytohormones is thought to affect the carrageenan yield of thallus from each seaweed variety: Lampung (42.77%), Tarakan (26.83%), and Kupang (22.63%) varieties. Sulistiani et al (2012) showed that tissue cultured seaweed seeds (F60) contained higher carrageenan (40.27%) than Tambalang cuttings (34.57%) and Maumere cuttings (34.26%) with an extraction method using KOH solvent. Likewise, the viscosity test results in this study ranged from 186.67 to 305 cP, with the lowest value recorded in the Tarakan variety and the highest in Lampung, while in the study of Sulistiani et al (2012), the lowest viscosity, of 173.6 cP, came from the carrageenan extraction of *K. alvarezii* from tissue culture (F60) and the highest was of 236.3 cP, from conventional sources (cutting). Testing of ash and silicate content (acid-soluble ash content) in this study was carried out to determine whether or not there was contamination of the test material during the analysis process; the value still met the SNI levels of pure carrageenan (SNI 2017).

Eigen analysis. Further testing (Table 3) resulted in the first principal component analysis (PCA) having a variance of 3.7717, or equal to 29% of the total variance of the actual data. The second PCA has a variance of 2.9820 with a proportion of 22.90% of the total variance of the data. The third PCA has a variance of 2.3274 with 17.90% of the total variance of the data. The fourth PCA has a variance of 1.6333 with a proportion of 12.60% of the total variance of the data, and the fifth PCA has a variance of 1.2368 with a ratio of 9.5%. The observations of Physico-chemical, phytohormones and final harvest weight contributed to four main components (eigenvectors) that explaining over 82.40% of the overall influence of variables on the total diversity (eigenvalues variance) of the data structure (along the eigenvectors), representing the seaweed sustainable productivity. The other components have a variance proportion of maximum 1.5, 0, with a total contribution of 10%, so the effect can be considered as negligible. The scree plot (Figure 2) also shows the diversity of data that describes the four components having an eigenvalue of 1.5 and adequately represents the data structure.

Eigen vector. Based on Table 4 (Eigen vectors) it can be seen that PC 1, PC2, PC3 and PC4 are above the other components (PC 5 to PC 10) and have an Eigen value variance of 1.5, with a variance proportion of 10%. PC1-PC4 values are 3.77, 2.98, 2.32 and 1.63, respectively (Figure 2). The linear combination of the first principal components is PC 1 =0.286 DO–0.358 TA (water temperature)-0.181 pH+0.050 salinity–0.461 IAA–0.198 gibberellin–0.439 zeatin+0.105 kinetin+0.255 ammonia+0.074 nitrite+0.175 nitrate +0.055 phosphate–0.443 weight. If we look at the data structure formed by PC1 there are positive and negative values that indicate the contribution of these parameters to the eigenvector (combination): DO (0.286), TA/water temperature (-0.358), pH (-0.181), salinity (0.050), IAA levels (-0.461), gibberellin (-0.198), zeatin (0.439), kinetin (0.105), ammonia (0.255), nitrite (0.074), nitrate (0.175), phosphate (0.055), and weight (-0.443). PC1 explains almost 29% of the data variance, compared to PC2 to PC4 which is only 22.9%, 17.9% and 12.6%, respectively. IAA, zeatin and water temperature (TA) had a significant contribution and were positively correlated to weight. Based on the biplot analysis (Figure 3), it can be observed that the vector of the weight variable coincides with the vector of the variables IAA, zeatin and TA. IAA stepwise regression analysis (sig<α) showed that IAA has an effect on the weight, with a coefficient value of 374.2, meaning that, for every increase in the IAA value by one unit, the estimated mean value of the weight increases by 374.2 units. Auxins in plants have complex effects in controlling growth, including cell and organ enlargement, root formation, maintaining apical dominance, stimulating vascular tissue differentiation, and triggering ethylene synthesis (Tarakhovskaya et al 2007).

Conclusions. *K. alvarezii* seedlings with different genetic sources (Lampung, Kupang and Tarakan) were correlated with the final harvest weight. In indoor seaweed seedlings of *K. alvarezii* Lampung, Kupang and Tarakan varieties, the DGR values obtained were 0.013±0.35% day^{-1}, -1.103±0.62% day^{-1}, and 0.707±0.23% day^{-1}, respectively. The highest DGR came from local non-tissue culture seedlings of the Tarakan variety, followed by Lampung variety's tissue culture (F60) and finally by the non-tissue culture seedlings of the Kupang variety. From the results of multivariate main component analysis (PCA) it results the first combination, representing 29% of the variability of the data structure: PC 1=0.286 DO–0.358 TA (water temperature)-0.181 pH+0.050 salinity–0.461 IAA–0.198 gibberellins–0.439 zeatin+0.105 kinetin+0.255 ammonia+0.074 nitrite+0.175 nitrate+0.055 phosphate–0.443 weight. Regression analysis shows that the IAA p-value is 0.002<α=0.05 which means that IAA affects weight. The relationship between IAA and weight can be seen from the regression equation: the IAA coefficient value is 374.2, which means that for every increase in the IAA value by one unit, the estimated mean weight value will increase by 374.2 units.

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