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Original research article

# Growth, Morphology and Growth Related Hormone Level in *Kappaphycus alvarezii* Produced by Mass Selection in Gorontalo Waters, Indonesia

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ABSTRACT

The use of high quality seed can support the success of the seaweed cultivation. This study was conducted to evaluate the growth performance, morphology and growth related hormone level of brown strain seaweed *Kappaphycus alvarezii* seed produced by mass selection. Selection was performed in the Tomini Gulf, Gorontalo, based on mass selection of seaweed seed protocol with a slight modification in cut-off 10% of the highest daily growth rate. Selection was carried out for four generations. The selected $4^{th}$ generation of seed was then used in cultivation performance test in the Celebes Sea, North Gorontalo, for three production cycles. The results showed that the selected *K. alvarezii* has higher clump weight and daily growth rate, longer thallus, more number of branches, and shorter internodes compared to the unselected control and seaweed from the farmer as external control. Furthermore, total sugar content, levels of kinetin hormone and kinetin:indole-3-acetic acid ratio were higher in selected seaweeds than that of unselected control and external control. Thus, mass selection method could be used to produce high growth of seed, and kinetin and indole-3-acetic acid play an important role in growth of *K. alvarezii*.



## 1. Introduction

*Kappaphycus alvarezii* (Doty) is a seaweed species of Rhodophyceae class (red algae) producing kappa carrageenan which is widely used in the food industry, pharmaceuticals, cosmetics, and others. The need for carrageenan products and raw materials of *K. alvarezii* is predicted to increase in the future. Farming of high quality *K. alvarezii* seed can increase the production level.

Seaweed seeds are generally obtained by some of the crop through vegetative propagation. Repeated vegetative propagation causes a decrease in genetic variability and resulting in decreased growth rate, carrageenan yield, gel strength and increase of susceptibility to disease (Hurtado and Cheney 2003). Therefore, a systematic attempt to generate a high quality seed is necessary.

Improvement of genetic quality can be performed by mass selection and tissue culture techniques. Tissue culture technique of seaweed have been developed by several research groups (Reddy *et al.* 2003; Kumar *et al.* 2004; Mulyaningrum *et al.* 2012; Sulistiani *et al.* 2012; Suryati *et al.* 2013). The success of acclimatization and field cultivation of seaweed *K. alvarezii* from somatic embryo culture has also been reported by Reddy *et al.* (2003) in India. Seaweed is monitored for seven generations and shows daily growth rate (DGR) of 1.5–1.8 times higher than that of the farmers (Reddy *et al.* 2003). In Indonesia, the success of field cultivation of *Gracilaria verrucosa* seed generated by tissue culture has been reported by Suryati *et al.* (2013). During the period of mass production, 1200 kg of seed is obtained in five cultivation cycles (Suryati *et al.* 2013).

On the other hand, mass selection methods have been found to increase yield in higher plants and improve the proportion of superior genotypes in a short time (Taran *et al.* 2004; Yang *et al.* 2013). The use of mass selection method to produce a high quality of *K. alvarezii* seed has been initiated by Pong-Masak *et al.* (2013). However, morphological and physiological characteristics of the

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selected seed remain to be examined. This study was performed to examine the growth, morphology and growth related hormone levels of the mass selected *K. alvarezii* brown strain with cut-off 10% of the highest DGR.

## 2. Materials and Methods

### 2.1. Seaweed source and mass selection procedure

*K. alvarezii* brown strain was collected from local farmers at Anggrek Waters, North Gorontalo Regency. Healthy, fresh, clean, brightly colored, highly branched, lush thalli and 30-day-old seaweed were chosen for mass selection. Selection was conducted in March to July 2013 in the Tomini Gulf, Boalemo Regency, Gorontalo (Figure 1).

Selection was conducted as described in seaweed selection protocols (RICA 2011) with slight modification in cut-off to 10% of highest DGR. Cultivation was performed for 30 days of each cycle, using long-line method. Cultivation construction was $50 \times 35$ m$^2$, containing 50 long ropes and each rope was 35 m in length, and the distance between the ropes was 1 m. Each rope contained 230 seed clumps with distance between clumps of 15 cm. Seaweed seed (G0) with initial clump weight of 50 g was tied to the rope. The seeds were placed at 30 cm from water surface. Seaweed clean-up activities of pests were done as needed.

Each clump was weighed at the end of each cultivation cycle. Seeds had 10% of highest DGR value were selected. The selected seed clumps were then cut into new clumps and tied to the new rope, and they were cultivated with the same methods, processes and period as previous cycles. Selected seed resulted from each cycle of mass selection was referred to a generation. In each cultivation cycle, there were internal control and external control. Internal control was unselected clumps that have DGR mean of population, while the external control was seeds from farmers around research location. Selection was carried out until 4$^{th}$ generation which usually had stabilized DGR and uniformity level about >90%.

### 2.2. Mass production and cultivation performance test

The 30-day-old selected seeds of 4$^{th}$ generation were used in mass cultivation to compare their growth performance, morphological and growth related hormone level with unselected internal control and seaweed from the farmers as external control. Mass production and cultivation performance test were conducted in the Celebes Sea, Anggrek, North Gorontalo Regency, in August to October 2013 (Figure 1). Cultivation performance test was performed for three cultivation cycles which 30 days for each cycle. Cultivation method was performed by the same procedures as applied in mass selection. Three ropes were prepared for each of the selected seeds, internal and external controls. Each rope contained 230 clumps of seed. Three samples were taken randomly to measure clump wet weight at the end of each cultivation cycle. Whole clump was used to analyze morphological measurements and total sugar content. Branch clumps were used to analyze hormone content.

### 2.3. Variables and statistical analysis

Morphological characteristics and physiological measurements were performed at the end of each cultivation cycle. Morphological characteristics measured were thallus length (the main thallus, branch I, II and III), internodes (primary and secondary), thallus diameter (primary, secondary and tertiary), branch numbers (I, II and III). Primary diameter is the diameter of the main thallus, secondary diameter is the diameter of the 1$^{st}$ branch, while the tertiary diameter is the diameter of the branches II and III (Figure 2). Physiological characteristics observed were DGR, total sugar, and hormone level of kinetin and indole-3-acetic acid (IAA). Total sugar content was estimated using the Luff Schoorl method, whereas kinetin and IAA hormone levels were estimated by spectrophotometric method (Unyayar *et al.* 1996).

Water qualities measured were *in situ* observation of temperature, dissolved oxygen, and salinity, while *ex situ* observation were $NO_3$ (nitrate), $PO_4$ (phosphate) and total organic matter. Rainfall as secondary data was obtained from the Gorontalo Meteorological Station.


**Figure 1.** Research locations for selection in Tomini Gulf and cultivation performance test in Celebes Sea, Gorontalo.

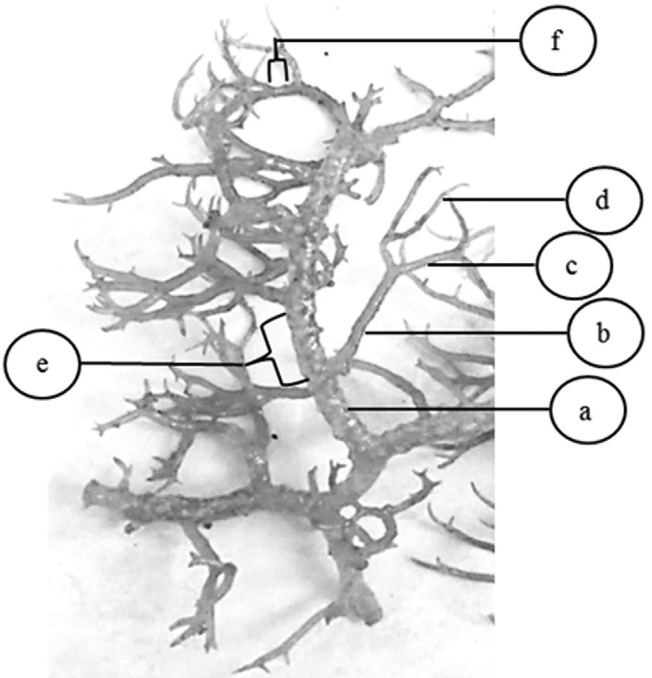


**Figure 2.** Morphology of seaweed: a) the main thallus, b) branch I, c) branch II, d) branch III, e) primary internodes, f) secondary internodes.

Morphological and physiological characteristics data were analyzed by analysis of variance at 5% significance level using software SPSS version 17. If there is a significant difference, analysis was continued using the Duncan multiple range test. Water qualities and rainfall data were analyzed descriptively.

## 3. Results

### 3.1. Growth performance

Mass selection is a simple method in selecting the best broods to produce the next generation. Seaweed mass selection can produce the best seeds for seaweed culture. In our previous study, DGR response at the 4$^{th}$ generation of selected seaweed was 2.22% − 5.21%. This DGR value showed improvement by 27.99% higher than the unselected internal controls, and 35.26% higher than the seaweed derived from the farmer as the external controls (RDISA 2013).

Mass production in this study aimed to expand the number of selected seeds to be cultivated at field level. The results showed that selected seaweed had higher biomass and DGR than the external and internal controls (Figure 3). Internal and external controls seeds did not grow due to a decline of the clump weight mean.

### 3.2. Morphological characteristics

The results showed that the total thallus length of the selected seaweed was 66.94% longer ($p < 0.05$) than the external control and 48.35% longer than the internal control (Table 1). Higher thallus length in the selected seaweed was significant contribution of branch II and branch III thalli length (Table 1). The total number of branches in the selected seaweed (316 branches) was 134.07% higher ($p < 0.05$) than external controls (135 branches) and 69.89% higher than internal control (186 branches). The increasing of branch number in the selected seaweed was contribution of branch II and III (Table 1). Selected and internal control seaweeds have shorter primary and secondary internodes than the external controls (Table 1; $p < 0.05$). Selected and internal control seeds showed thalli branches grow in cluster along the main thallus and branch I, that they look more lush clumps, whereas external control seaweed from the northern Gorontalo Waters has spreading branches (Figure 4). Furthermore, primary and secondary thallus diameters of three types of seaweed seed were not significantly different (Table 1; $p > 0.05$).

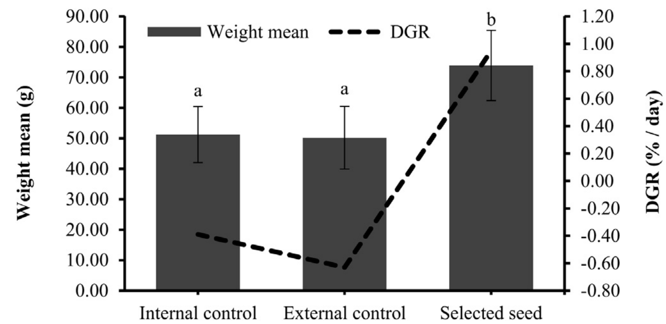


**Figure 3.** Clump weight mean and daily growth rate (DGR) of unselected internal control, farmer seaweed as the external control and the selected seeds.

### 3.3. Physiological characteristics

Total sugar content of selected seaweed was 15.52% higher than the internal control and 16.42% higher than external control ($p > 0.05$; Figure 5). Levels of kinetin and ratio of kinetin to IAA were higher in the selected seaweed (Figures 6A and B). Total sugar content, kinetin level and ratio of kinetin to IAA were in line with higher DGR in selected seed ($p < 0.05$).

### 3.4. Environmental conditions of the study site

Seasonal condition during mass selection conducted in March to July 2013 was the rainy season with monthly rainfall intensity range of 9.71−16.62 mm (Figure 7A). However, frequency of rain during cultivation performance test conducted from August to October 2013 began to decline. Even in September to the end of October there was no rain (Figure 7B). In cultivation performance test, some of water quality variables were not in optimum conditions for seaweed growth (Table 2). This led to the biofouling attachment of

Table 1. Thallus length, branches number, internodes and diameter of selected, internal control and external control seaweed seeds

| Variables | Seaweed seed | | |
|---|---|---|---|
| | Internal control, $n = 3$ | External control, $n = 3$ | Selected seed, $n = 3$ |
| **Thallus length (cm)** | | | |
| Main thallus | $15.10 \pm 2.71^a$ | $42.88 \pm 4.47^c$ | $28.63 \pm 4.16^b$ |
| Branch I | $102.39 \pm 14.12^a$ | $123.76 \pm 18.39^a$ | $177.65 \pm 45.99^a$ |
| Branch II | $155.49 \pm 23.90^a$ | $117.04 \pm 15.95^a$ | $229.01 \pm 26.68^b$ |
| Branch III | $83.60 \pm 12.81^b$ | $33.18 \pm 7.77^a$ | $93.70 \pm 15.53^b$ |
| Total | $356.58 \pm 44.60^a$ | $316.86 \pm 32.98^a$ | $528.98 \pm 76.53^b$ |
| **Branches number** | | | |
| Branch I | $34 \pm 3^a$ | $43 \pm 5^a$ | $57 \pm 13^a$ |
| Branch II | $70 \pm 7^a$ | $59 \pm 8^a$ | $154 \pm 45^b$ |
| Branch III | $82 \pm 12^b$ | $33 \pm 5^a$ | $105 \pm 25^b$ |
| Total | $186 \pm 21^{ab}$ | $135 \pm 11^a$ | $316 \pm 80^c$ |
| **Internodes length (cm)** | | | |
| Primary | $0.63 \pm 0.04^a$ | $1.20 \pm 0.09^b$ | $0.85 \pm 0.13^a$ |
| Secondary | $0.80 \pm 0.03^{ab}$ | $0.96 \pm 0.07^b$ | $0.72 \pm 0.07^a$ |
| **Thallus diameter (cm)** | | | |
| Primary | $0.66 \pm 0.03^a$ | $0.64 \pm 0.03^a$ | $0.73 \pm 0.03^a$ |
| Secondary | $0.41 \pm 0.03^a$ | $0.47 \pm 0.04^a$ | $0.44 \pm 0.03^a$ |
| Tertiary | $0.29 \pm 0.04^a$ | $0.28 \pm 0.03^a$ | $0.26 \pm 0.01^a$ |

Different superscript letters in the same line indicate significantly different (Duncan test; $p < 0.05$) and $n$ indicates number of sample.

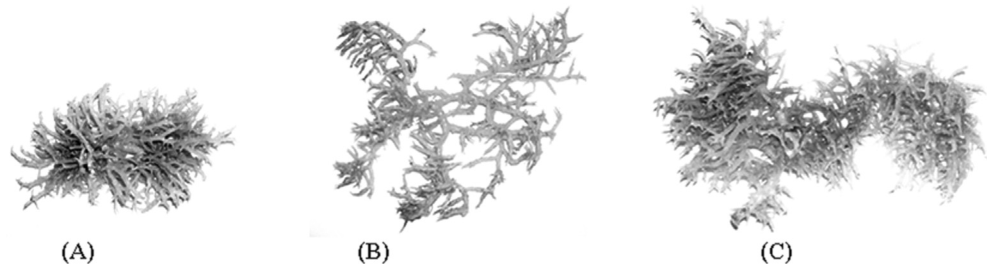


**Figure 4.** Performance of internal control (A), external control (B) and selected seaweed (C).

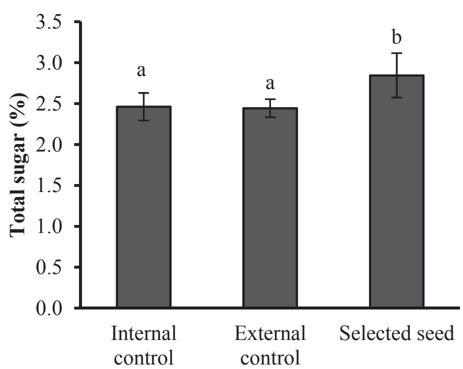


**Figure 5.** Total sugar content in internal control, external control and the selected seaweed. Internal control was unselected seaweed and external control was seaweed from the farmer around research location. Data with significant differences are indicated by different letters (Duncan test; $p < 0.05$).

barnacles and epiphytic algae to thalli surface in August. All thalli seeds also suffered from bleaching to pale yellow in September. In October, the thallus color became light brown.

## 4. Discussion

Biomass and DGR of all seaweed seeds show unfavorable growth in cultivation performance test. The thalli were attacked by biofouling and suffered from bleaching (whitening). This condition occurred because of water quality especially lower total phosphate and higher nitrate in August and September (Table 2). This phenomenon did not occur in mass selection activity.

The selected seeds obtained in this study had the longer thallus, more number of branches, but shorter in internodes than the controls. Selected seed and internal control look more like lush clumps, while external control has spreading branches. Presumably, selected and internal control seaweeds were affected by environmental conditions in the south Gorontalo Waters during mass selection.

Total sugar content of selected seaweed was higher than both controls. This result was in line with research of Pong-Masak *et al.* (2013) that carrageenan content in the selected seaweed was 6.07% and 12.72% higher than the internal control and external control, respectively. Carrageenans are sulfated linear polysaccharides of D-galactose and 3,6-anhydro-D-galactose extracted from certain Rhodophyceae seaweeds which is their main cell wall material (Campo *et al.* 2009). Generally, seaweed with higher DGR has higher carrageenan yield (Naguit *et al.* 2009). Carrageenan and biomass were strongly associated with the optimum harvest time. The optimum harvest time for *Kappaphycus* species is 8–9 weeks of cultivation (Villanueva *et al.* 2011). Thus, cultivation of selected seaweed seed has high potential to increase yield and carrageenan production.

Higher level of kinetin and ratio of kinetin to IAA in selected seaweed indicates that the two hormones play important role in the seaweed growth. Kinetin is a type of cytokinin, whereas IAA is a type of auxin. Root and shoot development in higher plant depends on the cytokinin:auxin ratio. Shoot formation was supported by high concentrations of cytokinin, whereas rooting was promoted by a high concentration of auxin (Brault and Maldiney 1999; Moubayidin *et al.* 2009). The presence of endogenous hormones such as auxin, cytokinin and abscisic acid in 11 species of red algae from the coast of Brazil has been reported by Yokoya (2010). Auxin and cytokinin had a regulatory role in the growth and morphogenesis *in vitro* in some species of red algae, such as *Gracilaria tenuistipitata, Gracilaria perplexa* (Yokoya *et al.* 2004), *Solieria filiformis* (Yokoya and Handro 2002).

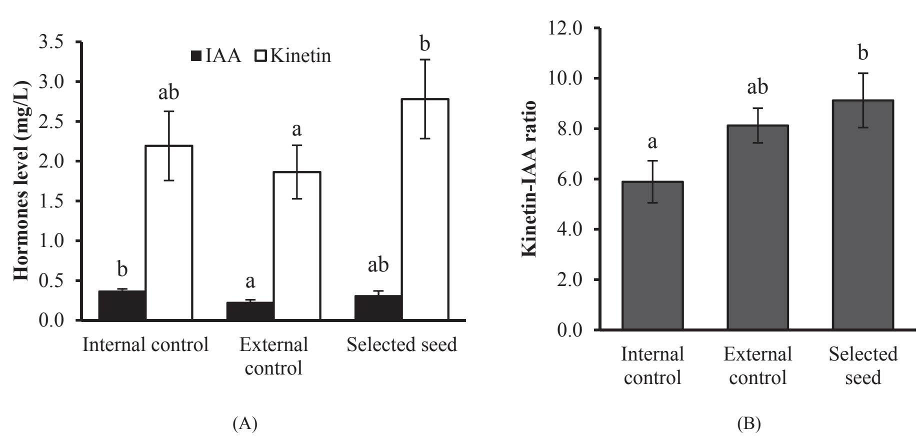


**Figure 6.** Hormone levels of indole acetic acid (IAA), kinetin (A), and kinetin-IAA ratio (B) in internal control, external control and selected seeds. Internal control was unselected seaweed and external control was seaweed from the farmer around research location. Different letters in the same bar type indicate significant difference (Duncan test; $p < 0.05$).

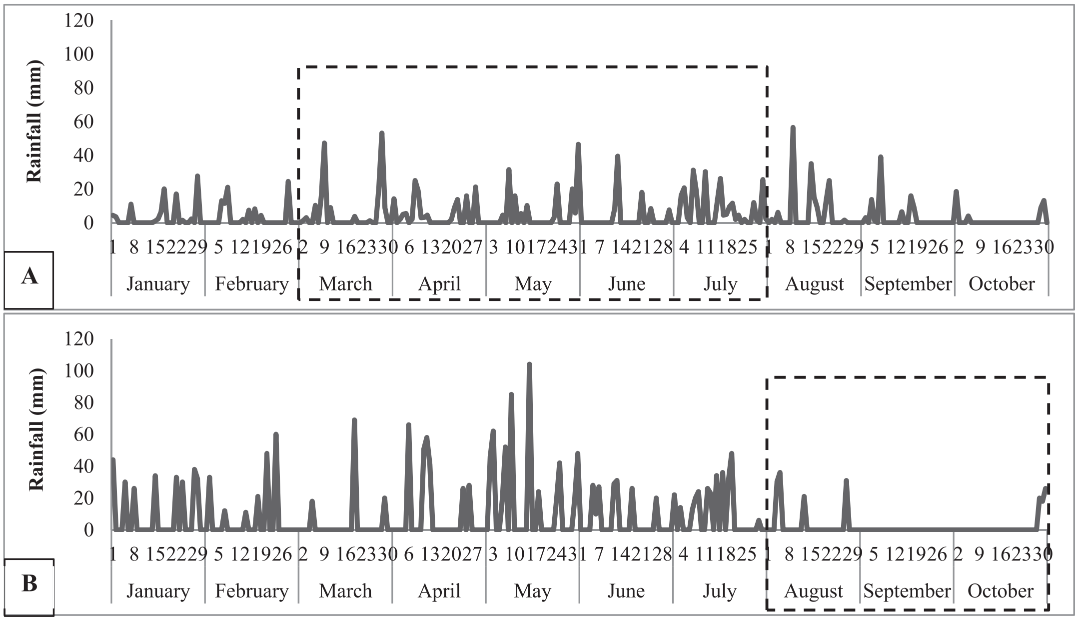


**Figure 7.** Rainfall level during *Kappaphycus alvarezii* mass selection (A) and cultivation performance test (B). The dashed lines indicate the study time. Image was processed from rainfall data of Tilamuta (A) and Kwandang stations (B), the Gorontalo meteorological station.

Physiologically, cytokinin controls cytokinesis (cell division) and bud development, whereas auxin induces elongation growth and apical dominance (Tarakhovskaya *et al.* 2007). Cytokinins play a role in mediating the response to extrinsic factors and response to biotic and abiotic stresses. This role together contributes to the regulation of quantitative growth in plants (Werner and Schmulling 2009). Basipetal auxin transport in the shoot actively inhibits bud development. Instead, acropetal transport of cytokinin was involved in the promotion of bud outgrowth (Ongaro and Leyser 2008).

Seaweed growth is affected by the environment (Glen and Doty 1990). Seasonal condition during mass selection was the rainy season with low rainfall intensity. Seaweed needs low rainfall. Therefore during mass selection, condition of seaweed was good, there was no disease and biofouling infected. It was caused by water quality that supported seaweed growth. However, in cultivation performance test, some of water quality variables were not supportable for seaweed growth. This led to the biofouling attachment and bleaching thalli seed in August and September, respectively. Bleaching thalli to pale yellow indicated as early of ice-ice disease. In October, the thallus color became light brown which indicated thalli began to recovery. Mizuta *et al.* (2002) investigated that seasonal changes affected pigment composition in two red algae, *Gloiopeltis furcata* and *Porphyra yezoensis*. Levels of chlorophyll a and phycoerythrin were high during the winter, but decreased in the late spring or summer. These changes were accompanied by changes in color from deep red to green or yellow, closely linked to fluctuations in phycoerythrin content. Phycoerythrin content was significantly correlated with the nitrogen content in both species (Mizuta *et al.* 2002).

Unfavorable condition in this study was consistent with Pong-Masak *et al.* (2009) that productive season of seaweed *K. alvarezii* in the North Gorontalo Waters was November to April, whereas in May to October, they were less productive. Because of the decline of water quality, biofouling attachment and ice-ice disease infection, seaweed DGR was <3% (Pong-Masak *et al.* 2009). In addition, our results also indicated that seaweed cultivation should not be performed during transition period from rainy season to dry season and in dry season. In that period, water quality declined especially higher nitrate and lower phosphate in August and September. Fried *et al.* (2003) conclude that if only one nutrient increases substantially the other nutrients will limit growth. If nitrogen is present in very high concentrations and low phosphorus levels, the phosphorus will be a limiting factor in the growth of algae (Fried *et al.* 2003). According to Correll (1998), phosphorus is an essential component of nucleic acids and many intermediary metabolites, such as sugar phosphates and adenosine phosphates, which are an integral part of the metabolism of all life forms. Although nitrogen is a component of proteins and found in many biological compounds (Rezael and Samimi 2013), Orbita (2013) found that growth and carrageenan content on thallus *K. alvarezii* positively correlated with water flow, phosphate and nitrate. Water flow during the rainy season increases the hydrodynamic thus increasing growth and carrageenan. In addition, phosphorus and nitrogen are nutrients that play an important role in the algae growth and carrageenan. Maximum carrageenan is obtained during the period of the highest nutrient supply (Orbita 2013).

Table 2. Water qualities of North Gorontalo and *Kappaphycus alvarezii* condition at cultivation performance test

| Variable | Unit | Month | | | Optimum level* |
|---|---|---|---|---|---|
| | | August | September | October | |
| **Water qualities** | | | | | |
| Temperature | °C | 28 | 29 | 30 | 24–30 |
| Dissolved oxygen | mg/L | 5.62 | 6.31 | 5.93 | >5 |
| Salinity | g/L | 36 | 33 | 34 | 28–34 |
| Total phosphate | mg/L | 0.03 | <0.002 | 0.15 | 0.10–0.20 |
| Nitrate | mg/L | 0.40 | 0.19 | 0.78 | 0.01–0.07 |
| Total organic matter | mg/L | 47.03 | 36.07 | 38.65 | |
| **Seaweed condition** | | | | | |
| Thallus color | | Dark brown | Pale yellow | Light brown | |
| *Biofouling* | | Barnacles; epiphytic algae | – | – | |

* Aslan (1998).

## Conflict of interest

There is no conflict of interest.

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