

Growth, Agar Yield and Water Quality Variables Affecting Mass Propagation of Tissue Cultured Seaweed *Gracilaria verrucosa* in Pond

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Abstract

Mass propagation of tissue culture produced seaweed seedling was conducted in the pond. The present study aims to evaluate the growth of tissue culture produced seaweed seedling of *Gracilaria verrucosa*, its agar yield and related water quality variables during propagation in pond. The seedling propagation was conducted in pond using long line method for 9 cycles (30 days per cycle). The daily growth rate was calculated by weighing 30% of the total amount of seaweed at ropes line every 15 days. Agar yield was analyzed every 30 days in hot water extraction method. Water quality monitoring was conducted every 15 days. The data of daily growth rate and agar yield were analyzed descriptively. A simple linear regression analysis was conducted in order to analyze the relationship between growth and agar yield as well relationship between water quality variables, growth and agar yield. The result showed that the average daily growth rate of nine cycles was $3.38 \pm 1.49\%$. The highest agar yield was yielded from cycle II ($27.84 \pm 1.60\%$) and the lowest was in cycle I (10.30 ± 2.15). There is no relationship between daily growth rate and agar yield ($R^2=0.055$). Daily growth rate was mostly influenced by phosphate ($P<0.05$), nitrate ($P<0.05$), salinity ($P<0.05$) and light penetration ($P<0.05$). Meanwhile, agar yield was mostly influenced by temperature ($P<0.05$) and light penetration ($P<0.05$). Present study promising for seaweed *G. verrucosa* mass cultivation of tissue culture product which is a feasible alternative method to supply seaweed seedlings in seaweed farming development.

Keywords: growth, agar yield, *G. verrucosa*, tissue culture, water quality

Introduction

Seaweed still dominates the production of the world's water plant commodity with 8% per year in recent ten years. As one of countries with great concern on tropical seaweed cultivation, Indonesia has huge contribution to increase production of world's water plants. The government policies in supporting seaweed cultivation significantly contribute to improve the global production of seaweed from 6.7% in 2005 to 36.9% in 2014. The production of Rhodophyte *Gracilaria* sp. increased during 2005–2014, from 936,000 tons to 3,752,000 tons (FAO, 2016). *G. verrucosa*, an exported potential seaweed species can be cultivated in ponds. There are some advantages of pond cultivation: water quality was easy to control and protected plant from several aquatic factors i.e: large waves, strong currents and predatory attacks. Production of *G. verrucosa* is continued to be increased. One strategy to provide sustainable and superior seedling is by seedling quality improvement through several methods such as clone selection (Pong-Masak et al., 2011), spore setting (Yudiati et al., 2004), and tissue culture with various techniques such as protoplast culture, somatic

embryogenesis and thallus fragment culture (Suryati et al., 2007; Suryati & Mulyaningrum, 2009; Mulyaningrum et al., 2013; Mulyaningrum et al., 2014). Tissue culture has been done on *Kappaphycus alvarezii* (Paula et al., 2001; Reddy et al., 2003; Muñoz et al., 2006); *Euclima denticulatum* (Hurtado-Ponce and Cheney, 2003); *Heterosiphonia japonica* (Husa and Sjøtun, 2006); and *Gelidiella acerosa* (Kumar et al., 2004). The main focus of tissue culture is for genetic improvement and clonal propagation for seaweed cultivation. Clonal propagation from simple thallus segment is the simplest vegetative propagation approach to select superior strain from wild population in order to improve quality of the seaweed. A number of studies have exploited organogenetic potential of seaweed to isolate superior clone of some high economic value species of seaweed. Eventhough this approach is considered successful, it needs a continuous monitoring and isolation of clone with superior quality for a certain characteristic as well as maintenance of selected strain. A massive production of tissue culture produced seaweed seedlings is absolutely needed to facilitate cultivation for the whole year (Reddy et al., 2008). The success of providing *G. verrucosa*

seedlings through tissue culture is inseparable from the success of propagation process in the ponds. Monitoring of growth, agar yield and water quality variables which affect the seedlings growth are essential to know the response of growth and agar yield of tissue culture *G. verrucosa* seedling to pond condition. The present study aims to evaluate the growth of tissue cultured produced seaweed seedling of *G. verrucosa*, its agar yield and related water quality variables during propagation in pond.

Materials and Methods

Mass propagation of tissue culture produced seaweed seedlings was conducted in experimental ponds in Taipa village, Takalar regency, South Sulawesi. *G. verrucosa* seedlings obtained from tissue culture according to the previous method (Mulyaningrum *et al.*, 2014) were propagated in the pond by applying long line method. Seedling with fresh thallus, a lot of branching and free of epiphytes was selected.

Thirty lines of rope were prepared as the equipment of cultivation. Each line has 7 meters long nylon rope with 5 mm diameter. Some pieces of plastic rope which functioned as strap for the seaweed clumps were tied up to the line with the distance between clumps was 15-20 cm. Twenty grams of seaweed seedlings was tied to each clump along the lines. Then, the lines were tied to bamboo stakes with distance between stakes was 50 cm, 15 cm from the bottom of the pond (Figure 1.). Nine cycles (30 d.cycle⁻¹) was carried out. During the cultivation, the lines were shaken to avoid epiphytes and mud. The water in the pond was changed during high tide. Monitoring of the growth of the seaweed and water quality was carried out every 15 days. Temperature, salinity, transparency, nitrate, phosphate and nitrite were measured and the 30% of total amount of the seaweed on the lines were weighed in every 15 days. The daily growth rate was calculated according to Dawes *et al.* (1993).

Agar yield was measured from triplicate random seaweed sample in 30 days. The sample was extracted in hot water, referred to the method of Angka and Suhartono (2000). Ten grams of dried seaweed was soaked in 0.25% chlorine solution for 3 x 24 hours. The seaweed was rinsed, cleaned and soaked in fresh water for 3 hours, then soaked in 10% sulfuric acid solution for 15 minutes; the sulfuric acid solution was then discarded and the seaweed was washed with fresh water and soaked in freshwater again for 15 minutes. After that, it was boiled in 500 ml of aquadest, let it cool, filtered and dried under the sunlight. Finally, the dried agar was weighed. and calculated according to Kumar and Fotedar (2009).

Growth and agar yield were analyzed descriptively and presented in graphics. A simple linear regression analysis was conducted in order to determine the relationship between growth and agar yield as well relationship between water quality variables, growth and agar yield.

Results and Discussion

Daily Growth Rate

Average daily growth rate of *G. verucossa* during cultivation in the pond was $3.38 \pm 1.49\%$. See Figure 2. The best and lowest average daily growth rate was occurred in cycle II ($5.03 \pm 1.39\%$) and VII ($1.22 \pm 0.78\%$). *G. verucossa* had good growth in cycle I–early VI with daily growth rate ranged 3.19–6.01%, but in the end of cycle VI–VIII it decreased to 0.66–2.89%. The growth started to increase again in cycle IX with daily growth rate ranged from 2.37% to 2.48%. The seaweed growth rate was greatly influenced by the weather during the cultivation which influenced the salinity of pond water. Cycles I until early VI (March until the beginning of August), the weather was relatively good with appropriate intensity of sunlight and rainfall for seaweed growth. Salinity of the pond was recorded at 4–28 ppt.



Figure 1. Mass propagation of tissue cultured seaweed seedling *G. verrucosa* using long line method

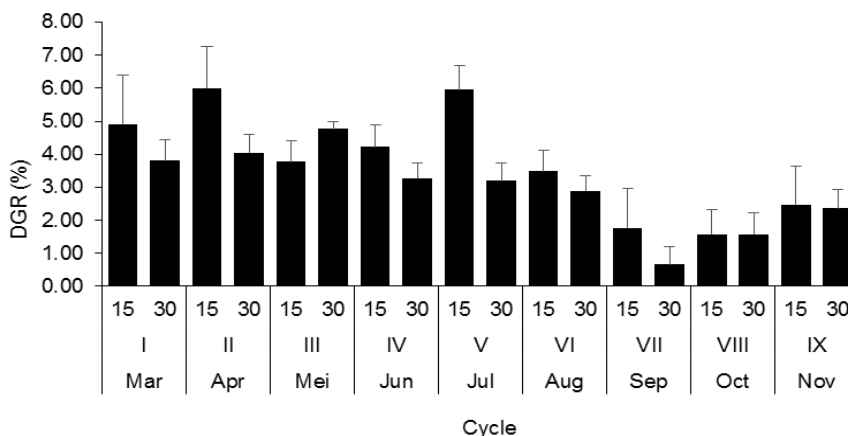


Figure 2. Growth of tissue culture produced *G.verrucosa* seedling in pond

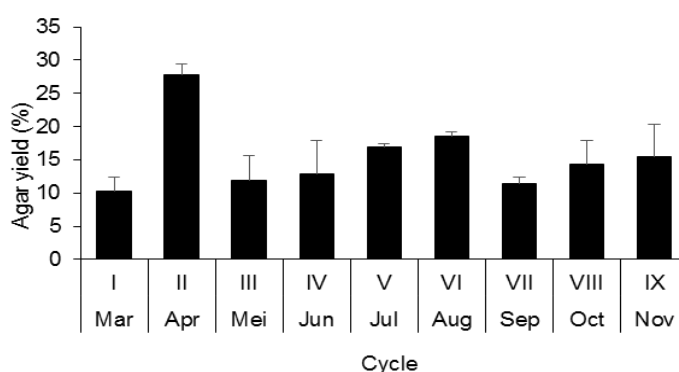


Figure 3. Agar yield tissue culture produced *G.verrucosa* seedling propagated in pond

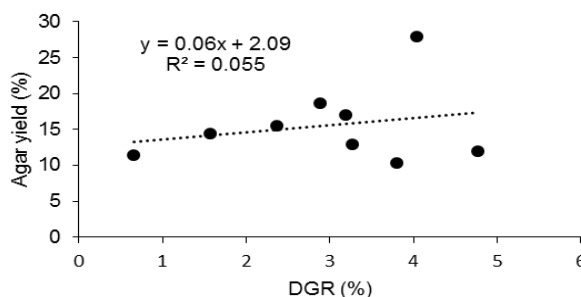


Figure 4. Relationship between growth and agar yield of seaweed tissue culture *G. verrucosa*

However, the dry season middle of August until October (end of cycle VI–VIII) increased the salinity to 38 ppt. Dry season also lead to decreasing supply of water so it was difficult to change the water pond. Daily growth rate has improved in cycle IX (started in November), when dry season changed into rainy season and the water salinity gradually decreased to 35–36 ppt. The daily growth rate has increased (Figure 2.). This is in accordance with Li-hong *et al.* (2002) which found that seaweed growth would be higher in low salinity.

The average growth of seaweed tissue culture was different from daily growth rate of *Gracilaria* sp.

which was obtained by Hurtado-Ponce *et al.* (1997). The daily growth rate of *Gracilaria* sp. was 4.5%. However, the growth is still considered well, which is corresponding to Mubarak *et al.* (1990) which explain that growth rate is considered advantageous if it is above 3%.

Agar yield

Average agar yield of tissue cultured seaweed seedlings in nine cycles was $15.50 \pm 5.35\%$. The highest yield was obtained in the second cycle ($27.84 \pm 1.60\%$) while the lowest yield was obtained in the first cycle ($10.30 \pm 2.15\%$) (Figure 3.). Agar is a carbohydrate of photosynthesis product, its

formation process is influenced by light intensity. The second cycle had optimum transparency and seaweed got enough sunlight for the process of photosynthesis so that the formation of agar was higher than other cycles. Orosco *et al.* (1992) and Oyieke (1993) reported the yield of marine cultured *G. verrucosa* was 8.1–30% and 14.7%, respectively.

Present study indicates no relationship between daily growth rate and agar yield of 30-day-old seaweed Figure 4 ($P < 0.05$). This result related to González-Gómez *et al.*, (1992) but that the growth of seaweed related to nutrient in the water. But study by Hayashi *et al.* (2007), showed that carrageenan yield of *Kappaphycus alvarezii* decreases when the nutrient content is high and growth and carrageenan yield has negative relationship.

Water quality

Generally, the condition in the pond during the research was conducive for seaweed growth, as presented in Table 1. The temperature of the water during the research was around 25–35°C. The lowest temperature was occurred in cycle I and the highest one was occurred in cycle II. Temperature effect on growth rate of some *Gracilaria* species have reported. Kim *et al.* (2002) reported the optimal temperature for *G. verrucosa* growth was 25°C. *G. cornea* showed a significant correlation between biomass and temperature, the optimum growth occurred when seawater temperatures were below 30°C (Orduña-Rojas & Robledo, 2002; Orduña-Rojas *et al.* 2002). Raikar *et al.*(2001) reported the optimum temperature for the growth of several *Gracilaria* species namely *G. arcuata*, *G. textorii*, *G. vermiculophylla*, *G. incurvata*, *G. foliifera*, *G. corticata*, *G. edulis* and *G. licheniodes*, found that the best temperature for the growth of *Gracilaria* spp. was around 20–35°C.

The light penetration during the research was normal (21–41 cm). The lowest light penetration was occurred in cycle VII and the highest one was occurred in cycle II. It corresponds with Boyd (1990) state that the range of transparency which is good for seaweed growth is around 10–40 cm. The range of water salinity during the research was quite wide

(4–38 ppt). However, the seaweed still could grow in those range of salinity. Good seaweed growth rate was occurred in cycle I-VI with salinity ranging in 4–28 ppt, while low growth rate was occurred in cycle VII-VIII in dry season with 35–38 ppt of salinity range. According to Choi *et al.* (2006), *G. verrucosa* can grow well in wide range of salinity (5–35 ppt) and optimally grow in salinity range of 15–25 ppt.

Nitrate content during the research was around 0.063–13.228 mg.L⁻¹. Nitrate content that supported seaweed growth rate was occurred in cycle I-VI and low nitrate content was occurred in cycle VII-VIII which led to low growth rate. Nitrate is considered limiting nutrient for algae growth if the content is less than phosphate in the water body. Nitrate is the best source of nitrogen for growth of several species of marine algae. Lack of nitrate is marked by bleaching of red algae thallus. The lowest tolerable content of nitrate for algae growth is 0.1 mg.L⁻¹, while the highest is 3 mg.L⁻¹ (Moore, 1991).

Phosphate content in the water during the research was around 0.002–0.445 mg.L⁻¹. The highest phosphate content was occurred in cycle II and the lowest one was occurred in cycle VII, this condition affected the growth rate of seaweed. Phosphate is also a main nutrient factor for algae. Phosphate is a dissolved compound in the water which contributes in protein formation and photosynthesis. According to phosphate content in the water, fertility rate of the pond can be categorized into three: low fertility rate has phosphate content of 0–0.02 mg.L⁻¹, medium fertility rate with phosphate content around 0.021–0.05 mg.L⁻¹ and high fertility rate with phosphate content 0.051–0.2 mg.L⁻¹. Meanwhile, nitrite content in water naturally is 0.001 mg.L⁻¹ and it is better not to contain nitrite more than 0.05 mg.L⁻¹ because it can be toxic for the water organisms (Moore, 1991; Effendi, 2003).

The result of simple regression analysis of water quality variable and daily growth rate indicate that phosphate ($R^2=0.84$; $r=0.917$; $P=0.000$), nitrate ($R^2=0.702$; $r=0.838$; $P=0.000$) salinity ($R^2=0.765$; $r=0.875$; $P=0.000$) and transparency ($R^2=0.460$; $r=0.678$; $P=0.002$), more significant

Table 1. Pond water quality variables during 9 cycles of seaweed tissue culture *G. verrucosa* cultivation (n=18).

Variable	Minimum	Maximum	Average	Standard deviation
Temperature (°C)	25	35	28.63	2.58
Salinity (ppt)	4	38	30	10.18
Transparency (cm)	24	41	24.94	5.66
Nitrate (mg.L ⁻¹)	0.063	13.228	2.55	3.80
Phosphate (mg.L ⁻¹)	0.002	0.445	0.16	0.12
Nitrite (mg.L ⁻¹)	0.001	0.047	0.01	0.01

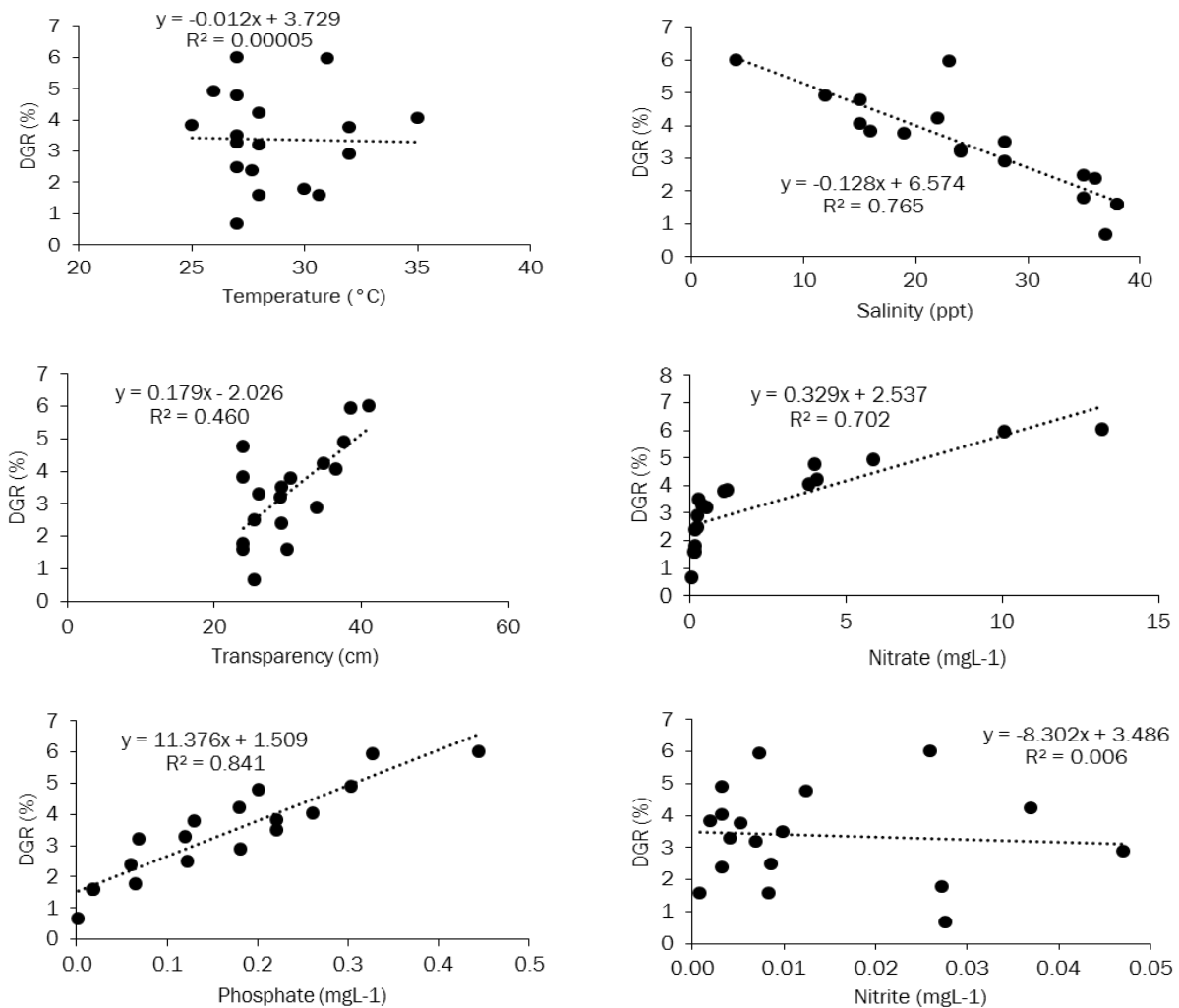


Figure 5. Relationship between daily growth rate of seaweed tissue culture produced *G. verrucosa* seedling and pond water quality variables

role in seaweed growth compared to other variables such as nitrite ($R^2=0.006$; $r=0.076$; $P=0.765$) and temperature ($R^2=0.00005$; $r=0.021$; $P=0.933$) as presented in Figure 5. Agar yield in seaweed is mostly influenced by temperature ($R^2=0.913$; $P<0.05$) and light transparency ($R^2=0.862$; $P<0.05$), but not by phosphate ($R^2=0.166$; $P>0.05$), nitrate ($R^2=0.150$; $P>0.05$), salinity ($R^2=0.056$; $P>0.05$), and nitrite ($R^2=0.0002$; $P>0.05$) as presented in Figure 6.

Water quality is a factor which influences the growth and agar yield of seaweed. Ak *et al.* (2011) reported that organic fertilizer with high nitrate content can boost the growth of *G. verrucosa* but it has negative relationship with agar yield. Araño *et al.* (2000) explain that light intensity and ammonium have significant influence to the growth and agar yield of seaweed both partially and simultaneously. Water quality variable, particularly temperature and transparency, play an important role in agar

formation. Agar yield in seaweed increased when the transparency increased. It is understandable since temperature and transparency influence photosynthesis which produces carbohydrate in form of agar (1999; Marinho-Soriano & Bourret, 2003).

Tissue culture with thallus fragment culture method produced from tissue cultured is a method of cultivation seedlings by vegetative clone propagation which has same characteristics with the parent. As an effort to improve the quality of seedlings produced by tissue culture, it is suggested to combine methods by selecting clone in propagation. Combination of tissue culture and molecular genetics can produce high quality seedlings (Baweja *et al.*, 2009). According to Thirumaran and Anantharaman (2009), vegetative propagation of seaweed is an effective way to massively produce seedlings. However, it is important to consider environmental factors since they greatly influence the growth and agar yield

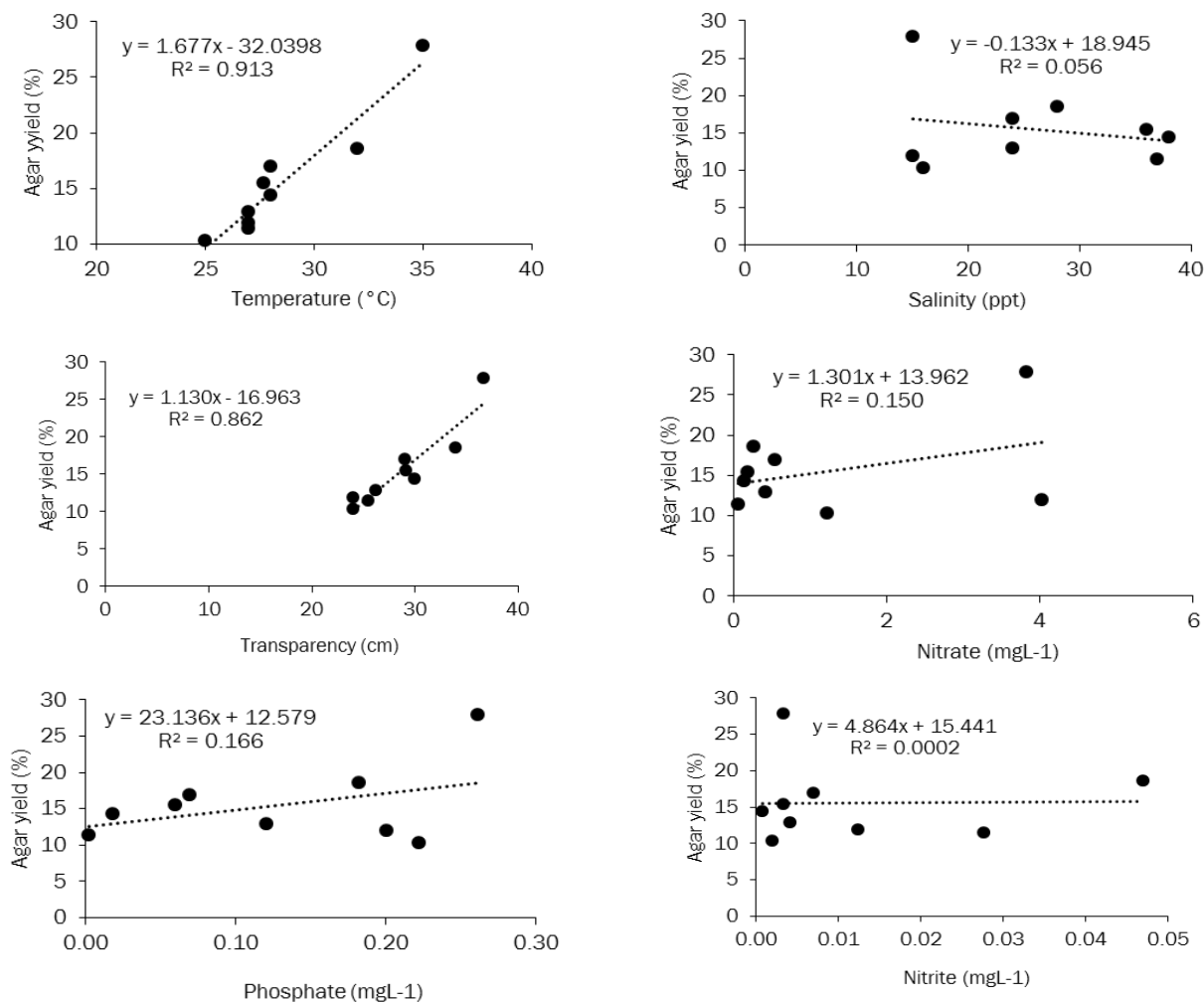


Figure 6. Relationship between agar yield of seaweed tissue culture *G. verrucosa* and pond water quality variables

produced (Chung *et al.*, 2007). The results of the present study suggested that the optimal condition for seaweed cultivation in Taipa village, Takalar regency, South Sulawesi were in March until the beginning of August, when the rain and light intensity support the growth of *G. verrucosa*. Meanwhile, the middle of August until October is the time when the condition is not optimal for seaweed growth because long dry season occurs in those months.

Conclusions

Tissue cultured produced seaweed seedling daily growth rate during nine cycles was $3.38 \pm 1.49\%$. The highest agar yield was yielded from cycle II ($27.84 \pm 1.60\%$) during April and the lowest was in cycle I (10.30 ± 2.15) during January. There is no relationship between daily growth rate and agar yield. Daily growth rate was mostly influenced by phosphate, nitrate, salinity and light penetration. Meanwhile, agar yield was mostly

influenced by temperature and light penetration. Tissue culture produced seedling seem as promising to supply the need for seaweed farming which is a feasible alternative method.

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