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Effect of grafting on the properties of *kappa*-carrageenan of the red seaweed *Kappaphycus alvarezii* (Doty) Doty ex Silva

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ABSTRACT

Kappaphycus alvarezii (*K. alvarezii*) of India water was grafted using different colored strains namely red (R), brown (B), and green (G) to produced four intra-generically grafted plants namely RB, RG, GB, and RGB, respectively. Gel strength of the *kappa*-carrageenan contents obtained from the parent strains was ranged 270–350 g cm^{-2}, while in the grafted strains it was significantly greater and was ranged 330–690 g cm^{-2}, respectively. Results of ANOVA analysis revealed significant variations in the growth rate of alga and gelling properties of *kappa*-carrageenan ($p < 0.05$), while principal component (PCA) analysis also indicated 89% of total variance in the gelling properties of carrageenan. In this paper, we reported the first data on the effect of grafting of different colored strains on the daily growth rate of *K. alvarezii* in the Indian brines, as well as yield and quality of the carrageenan produced.

1. Introduction

Food phycocolloids including carrageenans are commercially important hydrophilic colloids which are obtained from numerous species of red seaweed like *Chondrus crispus*, *C. ocellatus*, *Gigartina stellata*, *G. acicularis*, *G. pistillata*, *G. canaliculata*, *G. chamissoi*, *G. radula (also identified in the literature as Iridea species)*, *G. skottsbergii, Gymnogongrus furcellatus, Solieria chordalis*, *Calliblepharis jubata*, *C. ciliate*, *Cystoclonium purpureum, Kellymenia reniformis, Kappaphycus alvarezii, Eucheuma cottonii, E. spinosum, E. gelatinae, Furcellaria fastigiata, Hypnea musciformis, and H. spicifera* (Deslandes, Floc'h, Bodeau-Bellion, Brault, & Braud, 1985; Deslandes, Potin, Zinoun, & Folc'h, 1990; Doty, 1973; McHugh, 1987, 2003; Stanley, 1987).

The market for this phycocolloid has consistently grown at 5% per year, from 5500 tonnes in 1970 to up to 20,000 tonnes in 1995 (Bixler, 1996), in 2003 it was 35,000 MT/year with the value of around $300 million (McHugh, 2003). In recent years, the demand for carrageenan is on rise in the international market with an annual market at US$ 450 million (Rincones, 2010; Robledo & Freile-Pelrgrin, 2010). The carrageenan industry has become dominated by very large, multi-product companies with factories in Europe, US, Philippines, Chile, etc. Sales of this hydrocolloid in the US and Europe is holding up reasonably well despite the ongoing global recession.

For successful carrageenan industry, steady supply of raw material from reliable sources is essential to meet and maintain the required volumes and qualities. Select carrageenophytes (i.e. *Chondracathus canaliculatus*) have been exploited traditionally in Baja California and other places. However, the recent demand for carrageenan has necessitated the development of cultivation as well as search for promising resources mainly in the tropics (Robledo & Freile-Pelrgrin, 2010).

The carrageenophytes species including *Kappaphycus alvarezii* (Doty) Doty ex Silva, have continued as the tropical world's most important seaweed species and commercial farming of these seaweeds in several countries, mainly in the Southeast Asia and Africa, has been done through vegetative regeneration of the thalli (Bixler, 1996; White & Ohno, 1999). These are the key sources of carrageenan, which makes it as highly valuable seaweed sp. in all over the world (Bixler, 1996). Inure demand of *kappa*-carrageenan in the world market also makes *Kappaphycus* farming a worthwhile livelihood among the coastal fishing communities, as more than 1500; 80,000; and 400 fishing families in Indonesia, Philippines, and Republic of Kiribati, respectively, are generating a cash income from the cultivation of carrageenophytes (Hurtado, Agbayani, Sanares, & Castro-Mallare, 2001). As per the verbal communication ca. 800 fishing families in India are generating the cash

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income from the cultivation of *K. alvarezii* (Aqua Agri Pvt. Ltd., India).

Nowadays Philippines, Indonesia, Malaysia, Fiji and Tanzania are the main producers, with the selling quantities of about 1000 Mt (Munoz, Freile-Pelegrin, & Robledo, 2004). In the previous decades, the worldwide production of commercial eucheumatoid species have been increased from less than 1000 dry weight Mt to approximate of over 100,00 Mt, which are produced by 40,000–50,000 families (Doty & Alvarez, 1975; Ask & Azanza, 2002), but cultivated eucheumatoids appears incapable of meeting demand, at least in quality, price and volume for the requirements of the carrageenan processing industries (Ask, Batisbaga, Zertuche-Gonzalez, & de San, 2003). Numerous reports on the cultivation of eucheumatoid species are available but no more efforts have been made in the improvement of the quality of carrageenan. The existences of the green, red and brown colored strains of *K. alvarezii* have been reported in the literatures (Trono & Lluisma, 1992; Dawes, 1992). Seasonal growth rate and yields of carrageenan have been reported in the literatures, for different colored varieties of *K. alvarezii* and *E. denticulatum* (Azanza-Corrales & Sa-a, 1990; Dawes, Lluisma, & Trono, 1994; Munoz et al., 2004; Trono & Lluisma, 1992). Cheney, Ipswitch, and Wang (1994) have reported somatic hybridization, callus induction of *Eucheuma* spp. using *in vitro* method.

In this paper, we presented the first data about the effect of a novel grafting technique of different colored strains on the yield and quality of carrageenan produced from these grafted strains of *K. alvarezii* in the Indian seawaters.

2. Materials and methods

2.1. Materials

Three different colored strains of *K. alvarezii* namely red (R), brown (B) and green (G) were used in this study for grafting experiments under field conditions. Healthy plants were collected from the cultivation farm of CSMCRI, situated in the shallow waters of the Gulf of Mannar at Thonithurai, near Pamban Bridge (09°17′N, 79°2′E), Mandapam, India in the month of May 2005. Collected strains were brought to the laboratory for making the grafts.

2.2. Grafting

Selected fronds were washed thoroughly in sea water to remove the visible epiphytes and were cut obliquely in to the small pieces (ca. 8–10 cm) with the help of a blade (Fig. 2a and b). Grafts of two or more strains were made by placing two or more straws together; a portion of thallus of the one strain to be propagated was slipped onto the thallus of another strain, i.e. their corresponding "mates" and tied tightly (Fig. 2c and d). Similarly, grafts of four different combinations were made R + B, R + G, G + B and R + G + B, and were represented as RB, RG, GB and RGB, respectively. After tied tightly these collective plants were transferred in to the cultivation farm of CSMCRI, at Mandapam, for their proper attachment and increasing their biomass through modified protocol of cultivation (cf. Cheney et al., 1994). Raft method (1.5 m × 1.5 m size) was adopted for producing the large biomass of these strains. Daily growth rate (DGR%)

Fig. 1. (a) Marker pointing the attachment developed within different colored strains of *Kappaphycus alvarezii* through grafting method; (b) mature plants of grafted-RBG strains; and (c) mature plants of grafted-BG strains.

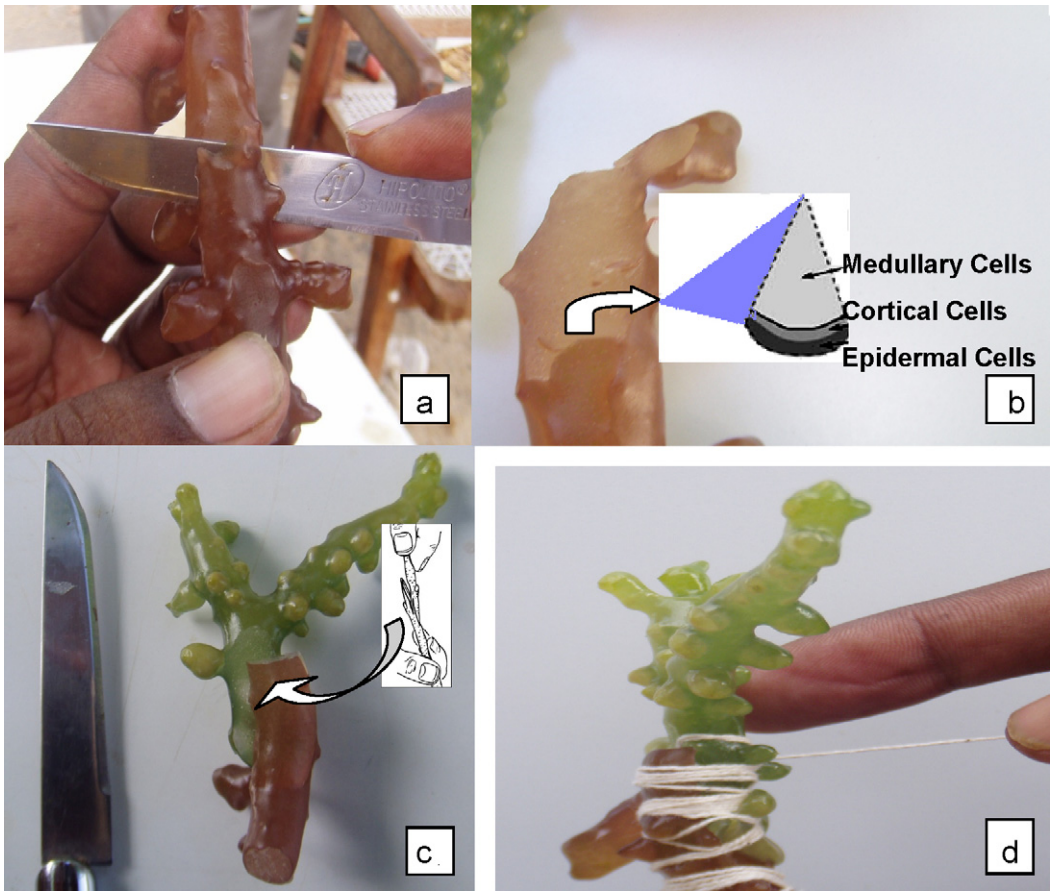

Fig. 2. Steps of grafting process: (a) oblique incision of the thallus; (b) cut-thalli showing area of epidermis, cortex and medulla; (c) a portion of thallus of the one strain to be propagated was slipped onto the thallus of another strain (arrow showing diagrammatic representation); and (d) corresponding "mates" tied tightly.

of the parent plant and grafted plants was recorded periodically, after 45 days from the date of planting following Eq. (1) (Dawes, Lluisma, & Trono, 1993).

$$\mathrm{DGR}(\%) = \ln(W_f/W_o)/t \times 100 \quad (1)$$

where W_f is the final fresh weight (in g) at t day; W_o is the initial fresh weight (in g); and t is the number of culture days.

Biomass yield Y (expressed as mean g fr wt/m) was determined using the following modified formula (Doty, 1986) to include the initial weight of the transplants, as given in the Eq. (2).

$$Y = \frac{(W_f - W_i)}{n} \quad (2)$$

where W_f is the final fresh weight (in g), W_i is the initial fresh weight (in g) and n is the number of the grafted plants.

2.3. Environmental factors

Samples of the seawater were collected from the cultivation farm of *K. alvarezii* on the monthly basis for examining the hydrobiological parameters. Seawater temperature was recorded in the definite time interval, using a standard centigrade thermometer. Salinity of the seawater of cultivation farm was measured using a Refractometer (ATAGO, Japan), and pH of seawaters was measured by pH meter (ELICO, India). Dissolved oxygen; inorganic PO_4–P; NO_3–N and NO_2–N were estimated using the method described by Strickland and Parsons (1972).

2.4. Extraction of kappa-carrageenan (kC)

The kC was extracted from the parent (R, B, and G) and grafted (RB, RG, GB, and RGB) plants in the laboratory scale, using a method described by Craigie and Leigh (1978). In brief, dry *K. alvarezii* sample (20 g dry each) was soaked in 0.5% Ca $(OH)_2$ solution for 2 h at room temperature. The soaked seaweed was then autoclaved at 107 °C for 1 h, after diluting with distilled water. Cooked seaweed was then homogenized in a kitchen grinder and treating the extractive with Celite up to the boiling and vacuum filtering the hot extractive over a Celite bed to obtain the clear extractive. The pH of the extractive was maintained ca. 7 with 0.1 M HCl. The solid product (kC) was then obtained by precipitating of the cleared extractive in iso-propanol (IPA) using 1:2 w/w, followed air and oven drying at 50 °C for 4 h.

2.5. Physical properties

The kC was powdered and used for various measurements. kC gel samples (1.0% gel) were prepared by dissolving in 1% KCl solution in an autoclave at 107 °C for 15 min. Gel strength measurements were done on a Gel Tester (Kiya Seisakusho, Ltd., Tokyo, Japan). Gelling and melting temperatures of gel samples were measured following the method described by Craigie and Leigh (1978). Apparent viscosity was measured on a Brookfield Viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072), using Spindle No. 1 at a speed of 60 rpm.

2.6. Chemical properties

The 3,6-anhydrogalactose was estimated by improved phenol–resorcinol method using fructose as standard (Yaphe & Arsenault, 1965). Metal ions and sulphate contents analyses (ICP) were carried out on a Perkin-Elmer ICP-OES Optima 2000DV machine following the method described in our previous work (Wolnik, 1988; Meena et al., 2007). Elemental analysis (e.g. C, H, N), apparent viscosity and ash contents were carried as described in our previous work (Meena, Prasad, & Siddhanta, 2006; Meena et al., 2007). Total sugar contents were determined by the phenol-sulfuric acid as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956).

2.7. FTIR–ATR analysis

FTIR-ATR spectra of *kappa*-carrageenan samples prepared from grafted and ungrafted seaweed samples were analyzed using a Perkin-Elmer (Vector 33, USA). The ATR technique was used and samples were dried in vacuum before analysis at 50 °C for 4 h. The characteristics IR peaks of carrageenan polymer (of RBG) (in the range of 1500–600 cm^{-1}) have been shown in Fig. 7, and using commercially available *kappa*-carrageenan (M/s Carl Roth GmbH, Germany) as the reference sample (Pereira, Amado, Critchley, van de Velde, & Ribeiro-Claro, 2009). IR spectra of kC obtained from other strains are identical with that of the reported IR spectra of RBG, herein.

2.8. Statistical analysis

Analysis of variance (ANOVA) was done to find the variation in DGR, biomass yield and gelling properties by using SYSTAT version 7.0. To carry out the analysis of the variance (ANOVA) four replications ($n = 4$) of each parameter in three groups were made. Software UNSCRAMBLE version 9.8, Principal Component Analysis was done to determine the variations in the gel properties.

3. Results

3.1. Grafting

The physical attachment of the different colored strains during grafting in the cultivation farm confirmed the feasibility of this technique in these strains (Fig. 1a). The duration of attachment in the blends was depend on the colors of the strains, and was varied significantly. The physical attachment in the GB and RGB groups was observed after 21 and 25 days from the date of initial plantation, respectively. The results of the present study revealed that the different colored strains, e.g. R, B, and G of *K. alvarezii* may be attached physically under the ambient conditions of the Indian seawaters at the south east coast (Mandapam, TamilNadu) of India. This result indicated that physically attached different colored strains of *K. alvarezii* could be produced under the field conditions of the Indian waters.

Similarly, the DGR and yields of biomass of *K. alvarezii* were depends on the color of the strain, and the biomass yield and the DGR were significantly different in the different colored strain. Generally, the DGR and biomass yields were improved considerably when grafted the different colored strains in the cultivation farm. ANOVA and PCA results revealed that DGR and biomass yields of *K. alvarezii* were significantly greater in the grafted strains than those of un-grafted strains of *K. alvarezii* ($p < 0.05$). This result indicated that this simple grafting technique applied on the field level at south east coast of India could be used for physical blending and increasing the biomass of the commercially important red seaweed *K. alvarezii*, which is the main raw material for producing the commercially important phycocolloid namely carrageenan.

The growth rate of the biomass was significantly greater in all the grafted strains, e.g. RG, BG, RB and RGB than those of the un-grafted, e.g. R, B and G strains. The biomass yield of un-grafted R strain was slightly greater 100 g fr wt/m than those of un-grafted G and B (ca. 90 g fr wt/m), after 45 days of plantation. The greatest and lowest biomass yields 350 g fr wt/m and 200 g fr wt/m were obtained in the grafted RG and BG strains, respectively, while the biomass yields were 310 g fr wt/m and 210 g fr wt/m for RGB and RB plants, respectively, after 45 days of plantation. The intact grown plants of RGB and BG have been shown in Fig. 1b and c. The line plot made on biomass showed distinct difference between the un-grafted and the grafted samples (Fig. 6a).

DGR of the parent colored strains of *K. alvarezii* was increased after grafting in the groups (Table 2). DGR of un-grafted R, B and G strains of *K. alvarezii* was observed between 6.10% day^{-1} to 7.24% day^{-1}. The greatest and lowest DGR 7.24% day^{-1} and 6.10% day^{-1} was obtained for un-grafted B and G strains, respectively. The greatest and lowest DGR 8.19% day^{-1} and 6.4% day^{-1} were obtained in the grafted RG and BG strains, respectively (Table 2). The DGR of *K. alvarezii* was also enhanced significantly in RB and RBG blends and were 7.06% day^{-1} and 7.96% day^{-1}, respectively (Table 2). The ANOVA results revealed that the DGR and biomass production were significantly greater in grafted strains than those of the parent R, B, and G strains of *K. alvarezii* ($p < 0.05$) (Table 2).

3.2. Environmental factors

Seawater parameters from the cultivation farm were recorded during the cultivation of *K. alvarezii* (Table 1). The experimental area has a confluence of both Gulf of Mannar and Palk Bay waters. The temperature of the seawater in the cultivation farm was ranged 24 ± 0.5 °C to 29 ± 0.5 °C during this study period. The greatest 29 ± 0.5 °C and the lowest 24 ± 0.5 °C seawater temperatures were observed in the month of May and December, respectively, from the cultivation farm. The salinity of the cultivation farm was ranged 28 ± 1.0 ppt to 35 ± 1.0 ppt during the study period. The greatest 35 ± 1.0 ppt and lowest 28 ± 1.0 ppt salinity values were obtained in the month of July/March and December, respectively (Table 1). Dissolved oxygen and pH content of the seawater collected from cultivated farm was ranged 0.8 ± 0.01 to1.6 ± 0.01 mg l^{-1} and 7.7 ± 0.1 to 8.2 ± 0.1 during the cultivation of *K. alvarezii*, respectively. Nitrite, nitrate and phosphate contents of the seawater samples collected from the cultivation site were ranged 0.12 ± 0.01 to 6.18 ± 0.1 μM; 0.008 ± 0.001 to 0.45 ± 0.01 μM and $0.38 \pm 0.01$to 6.46 ± 0.1 μM, respectively (Table 1). The results of this study revealed seasonal variation in the seawater parameters of the cultivation site.

3.3. Yield (%)

Yields were calculated on the basis of as received dry seaweed containing nil moisture. The yield of *kappa*-carrageenan (kC) obtained from grafted and un-grafted strains was ranged 30 ± 1.0% to 39 ± 1.5 (Table 3). The yield of kC was increased when grafting the different colored *K. alvarezii* under the identical conditions. The lowest 30 ± 0.5% and greatest 35 ± 1.5% yields of kC were obtained from the un-grafted G and B strains, respectively, while the lowest 31 ± 0.5% and greatest 39 ± 1.5% yields of kC were obtained from the grafted RB and RBG strains, respectively. The similar trends were observed for the biomass growth of the plants (Table 2). ANOVA analysis results indicated significant variation in the yields of kC obtained from the grafted and un-grafted *K. alvarezii* strains ($p > 0.05$).

Table 1
Seasonal variations in the environmental parameters recorded during this study period (±SD).

Month	pH	DO	Temperature (°C)		Salinity	Nitrite	Nitrate	Phosphate
			Air	Water				
May 2005	7.98 (±0.1)	1.22(±0.01)	31.5 (±0.5)	32 .0 (±0.5)	33 (±0.5)	0.27 (±0.01)	0.104 (±0.001)	4.52 (±0.1)
June 2005	8.04 (±0.1)	1.03(±0.01)	30.0 (±0.5)	27.5 (±0.5)	34 (±0.3)	0.45 (±0.01)	0.008 (±0.001)	6.46 (±0.1)
July 2005	7.88 (±0.1)	0.91(±0.01)	27.5 (±0.5)	26.5 (±0.5)	35 (±1.0)	0.42 (±0.01)	0.026 (±0.001)	4.64 (±0.1)
August 2005	7.80 (±0.1)	0.9 (±0.01)	29.0 (±0.5)	27.0 (±0.5)	33 (±0.5)	1.2 (±0.01)	0.029 (±0.001)	3.05 (±0.1)
September 2005	7.90 (±0.1)	1.14(±0 .01)	28.5 (±0.5)	28.0 (±0.5)	33 (±0.2)	0.46 (±0.01)	0.241 (±0.001)	1.81 (±0.01)
October 2005	7.71 (±0.1)	0.80(±0.01)	27.0 (±0.5)	26.5 (±0.5)	32 (±0.5)	0.42 (±0.01)	0.064 (±0.001)	1.81 (±0.01)
November 2005	7.88 (±0.1)	1.59(±0.01)	27.5 (±0.5)	24.5 (±0.5)	30 (±0.4)	0.41 (±0.01)	0.012 (±0.001)	1.85 (±0.01)
December 2005	8.00 (±0.1)	0.91(±0.01)	26.0 (±0.5)	24.0 (±0.5)	28 (±1.0)	0.13 (±0.01)	0.211 (±0.001)	0.38 (±0.01)
January 2006	8.23 (±0.1)	1.26(±0.01)	29.0 (±0.5)	26.5 (±0.5)	31 (±0.3)	1.78 (±0.1)	0.45 (±0.01)	0.43 (±0.01)
February 2006	8.19 (±0.1)	1.60(±0.01)	29.0 (±0.5)	27.0 (±0.5)	33 (±0.4)	0.62 (±0.01)	0.18 (±0.01)	1.62 (±0.01)
March 2006	8.1 (±0.1)	0.91(±0.01)	29.0 (±0.5)	26.5 (±0.5)	35 (±0.2)	6.18 (±0.1)	0.27 (±0.01)	0.96 (±0.01)

Table 2
Variations in DGR between grafted and un-grafted samples.

	1	2	3	4	5	6	7
1	1.000						
2	**0.026***	1.000					
3	0.988	**0.005****	1.000				
4	0.546	0.606	0.185	1.000			
5	**0.000*****	**0.013***	**0.000*****	**0.000*****	1.000		
6	1.000	**0.042***	0.957	**0.000*****	0.678	1.000	
7	**0.001****	0.789	**0.000*****	0.230	0.063	**0.002****	1.000

1 = R, 2 = B, 3 = G, 4 = RB, 5 = RG, 6 = BG and 7 = RGB.
Values with a significance level <5% are marked in bold.
* $p = 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

3.4. Physical properties

The lowest gelling 48 ± 0.5 °C and melting 70 ± 0.5 °C temperatures were exhibited by the kC gel samples prepared from the RG and R strains, respectively, while greatest gelling 54 ± 0.5 °C and melting 77 ± 0.5 °C temperatures were exhibited by the kC gel samples obtained from the RBG strain (Table 3). Apparent viscosities of the kC gel samples prepared from the grafted and un-grafted strains were ranged 45 ± 0.5 cP to 58 ± 1.0 cP in 1.0% sol at 80 °C. The greatest 58 ± 1.0 cP and lowest 45 ± 0.5 cP were exhibited by the gel samples prepared from RB and G strains, respectively. The gel strength of kC was ranged 270 ± 20 g cm^{-2} to 350 ± 20 g cm^{-2} in the gel samples prepared from the un-grafted R, B and G strains. Gel strength of kC gel samples was significantly greater in the grafted strains and ranged 330 ± 25 g cm^{-2} to 690 ± 25 g cm^{-2}. The lowest gel strength 270 ± 20 g cm^{-2} and 330 ± 25 g cm^{-2} was obtained from the un-grafted G strain and grafted RG strain, while greatest 350 ± 20 g cm^{-2} and 690 ± 25 g cm^{-2} was obtained from the un-grafted R and grafted RB strains, respectively (Table 3). The ANOVA analysis revealed that the gel strength of kC obtained from the grafted strains was significantly higher than those of the un-grafted strains ($p < 0.05$) (Table 3). Similarly, this can also distinguished diagrammatically from the line plot (Fig. 6 b). This result indicated that grafted strains of *K. alvarezii* could be farmed for large scale propagation, which produced superior quality of kC. The result of ash content revealed that the ash contents were higher in grafted strains than those of the un-grafted strains (Table 3). The lowest 15.24 ± 0.5% and greatest 25.37 ± 0.5% ash contents were obtained in the kC samples obtained from grafted RG and RB strains, respectively (Table 3).

3.5. Chemical properties

The 3,6-anhydrogalactose (AG) content in the kC samples was ranged 22.0 ± 0.5% to 39.0 ± 1.0% (Table 3). The minimum 22.0 ± 0.5% and maximum 39.0 ± 1.0% 3,6-AG contents were obtained from the kC samples of the un-grafted R and grafted RG strains, respectively. ANOVA analysis revealed that the 3,6-AG contents were significantly greater in kC samples obtained from the grafted strains than those of the un-grafted strains. The greater gel strength values of the kC gel samples obtained from the grafted strains can be explained on the basis of higher 3,6-AG values for the grafted strains than those of un-grafted strains (Table 3). The sugar contents of the un-grafted strains were significantly decreased after grafting in different combinations (Table 3). Total sugar content was also dependence on the color of the strains and was ranged 33.5 ± 1.0% to 47.0 ± 1.0% (Table 3). The minimum 33.5 ± 1.0% and maximum 47.0 ± 1.0% sugar contents were obtained from the grafted RB and un-grafted B strains, respectively. The sulphate content was decreased in grafted samples when compared to the un-grafted plants, and was ranged 15.0 ± 0.4% to 18.7 ± 0.2%. The lowest 15.0 ± 0.4% and the greatest 18.7 ± 0.2% was obtained from the kC samples prepared from the grafted RG and un-grafted G strains, respectively. ANOVA analysis revealed that the sulphate, 3,6-AG, gel strength and sugar contents of the grafted strains were significantly different from the un-grafted strains ($p < 0.05$). The elemental analysis (C, H, N, S) result revealed that their was no significant variation in the values of C and H obtained from the kC samples of grafted and un-grafted strains (Table 4), while N is not present in all kC samples obtained from grafted and un-grafted strains. Furthermore, the S content of the

Table 3
Properties of *kappa*-carrageenan prepared from grafted and un-grafted *Kappaphycus alvarezii* samples* (±SD).

Sample codes	Yield	G/S	G/T	M/T	Ash	Sul	3,6-AG	Total Sugar
NS-I-26-1 Green (G)	30 ± 1.0	270 ± 20	52 ± 0.5	71 ± 0.5	18.5 ± 0.2	18.7 ± 0.2	25.0 ± 0.5	42.5 ± 1.5
NS-I-26-2 Brown (B)	35 ± 1.5	330 ± 10	50 ± 0.5	72 ± 0.5	20.6 ± 0.3	17.3 ± 0.3	31.0 ± 1.0	47.0 ± 1.0
NS-I-26-3 Red (R)	34 ± 0.5	350 ± 20	50 ± 0.5	70 ± 0.5	21.2 ± 0.5	18.1 ± 0.1	22.0 ± 0.5	44.5 ± 1.5
NS-I-26-4 Graft RG	37 ± 1.5	330 ± 25	48 ± 0.5	70 ± 0.5	15.24 ± 0.5	15.0 ± 0.4	32.0 ± 1.0	40.0 ± 1.5
NS-I-26-6 Graft RB	31 ± 0.5	690 ± 25	52 ± 0.5	75 ± 0.5	25.37 ± 0.5	15.1 ± 0.2	39.0 ± 1.0	33.5 ± 1.0
NS-I-26-8 Graft BG	32 ± 1.0	560 ± 40	53 ± 0.5	76 ± 0.5	20.5 ± 0.3	16.9 ± 0.1	37.5 ± 1.5	35.0 ± 1.0
NS-I-26-10 Graft RBG	39 ± 1.5	540 ± 30	54 ± 0.5	77 ± 0.5	19.45 ± 0.3	17.0 ± 0.2	37.5 ± 0.5	36.0 ± 0.5

* Sample codes.

Table 4
Elemental analysis and apparent viscosity of *kappa*-carrageenan obtained from grafted and un-grafted *Kappaphycus alvarezii* samples* (±SD).

Sample codes	C (%)	H (%)	S (%)	Apparent Viscosity (cPs)[a]
NS-I-26-1 Green (G)	26.88 ± 0.5	4.20 ± 0.2	6.4 ± 0.1	45 ± 1.0
NS-I-26-2 Brown (B)	26.70 ± 0.1	4.40 ± 0.2	6.3 ± 0.2	53 ± 1.0
NS-I-26-3 Red (R)	26.93 ± 0.3	4.01 ± 0.5	6.0 ± 0.1	48 ± 1.0
NS-I-26-4 Graft RG	25.39 ± 0.1	3.84 ± 0.2	5.6 ± 0.2	49 ± 1.0
NS-I-26-6 Graft RB	26.11 ± 0.2	3.86 ± 1.0	5.4 ± 0.3	58 ± 1.0
NS-I-26-8 Graft BG	25.92 ± 0.1	4.12 ± 0.2	5.6 ± 0.2	57 ± 1.0
NS-I-26-10 Graft RBG	27.01 ± 0.1	3.75 ± 0.1	5.8 ± 0.2	52 ± 1.0

* Sample codes.
[a] Measured at 80 °C in 1% sol.

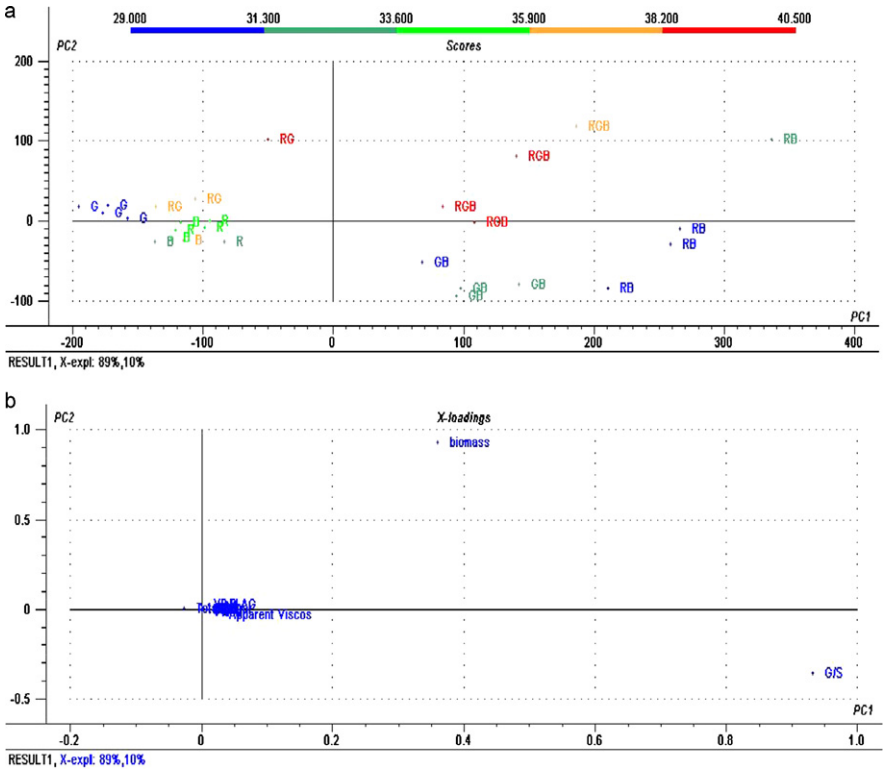

Fig. 3. PCA showing variation among the grafted and the un-grafted plants: (a) score plot; (b) loading plot on gel properties.

kC samples was significantly different in the grafted and un-grafted strains, and ranging 5.4 ± 0.1% to 6.4 ± 0.1%. The lowest 5.4 ± 0.1% and greatest 6.4 ± 0.3% S contents were obtained in the kC samples obtained from RB and G strains, respectively.

3.6. Principal component analysis (PCA)

The PCA was made using all characters of the gel extracted from the different un-grafted and grafted strains. The score and loading plot of PCA explained 89% of total variance among the grafted plants and the un-grafted. The gel strength showed greater difference among all the variables (Fig. 3). The correlation loading plot showed that gel strength and melting temperature have an extreme position to the right of the plot along PC1. They were closed to each other and far from the centre, very close to the 100% explained variance circle (i.e. the outer ellipse), they correlates negatively with sugar content which lies at the extreme opposite position of the same axis. Similar, relation was found between the biomass, viscosity and the ash content (Fig. 4). The DGR, yield, sulphate, 3,6-AG, gel temperature and the elemental properties (C, H, S) lie in the inner ellipse, i.e. close to the 50% of variance circle. They were near to the centre and showed weak correlation between the yield and the sulphate content. The mean and standard deviation plot (Fig. 5a and b) showed greater value for gel strength (skewness: 0.475662, kurtosis: −1.344251, mean: 438.5714, variance: 22283.07 and standard deviation: 149.2751).

3.7. Spectral characterization

Fig. 7 presents the FTIR–ATR spectra of *kappa*-carrageenan, of *K. alvarezii* (obtained from RBG) and of commercially available *kappa*-carrageenan (M/s Carl Roth GmbH, Germany). The spectra

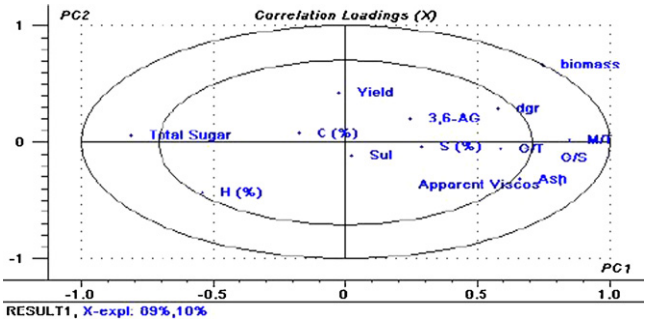

Fig. 4. Plot showing relationship among the gel properties.

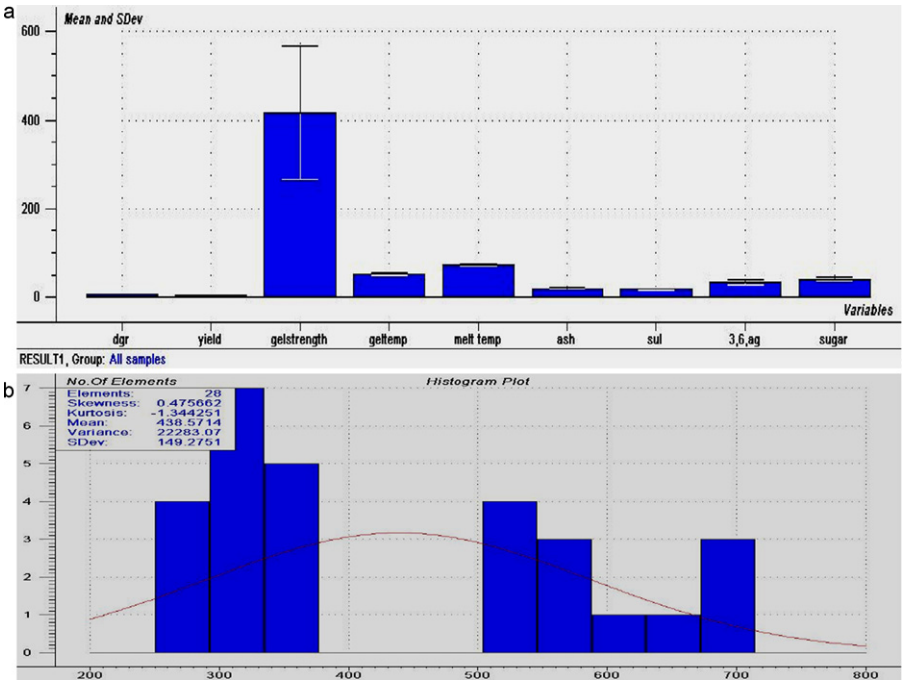

Fig. 5. (a) Mean and standard deviation among gel properties; and (b) histogram plot showing the values of mean and standard deviation of gel strength.

of these samples show characteristics band of *kappa*-carrageenan at approximately 845 cm^{-1}. No presence of IR band at around 805 cm^{-1}, a characteristic band of the *iota*-carrageenan indicating the absence of this form. The presence of 3,6-anhydro-D-galactose in the sample is confirmed by the occurrence of a strong absorption band at approximately 930 cm^{-1} (Pereira et al., 2009).

4. Discussion

Strain improvement in eucheumatoid cultivation focuses on different goals, e.g. higher growth rate of biomass, which may produced greater yield and superior quality of *kappa*-carrageenan. This could be made through selection of wild plants, breeding pro-

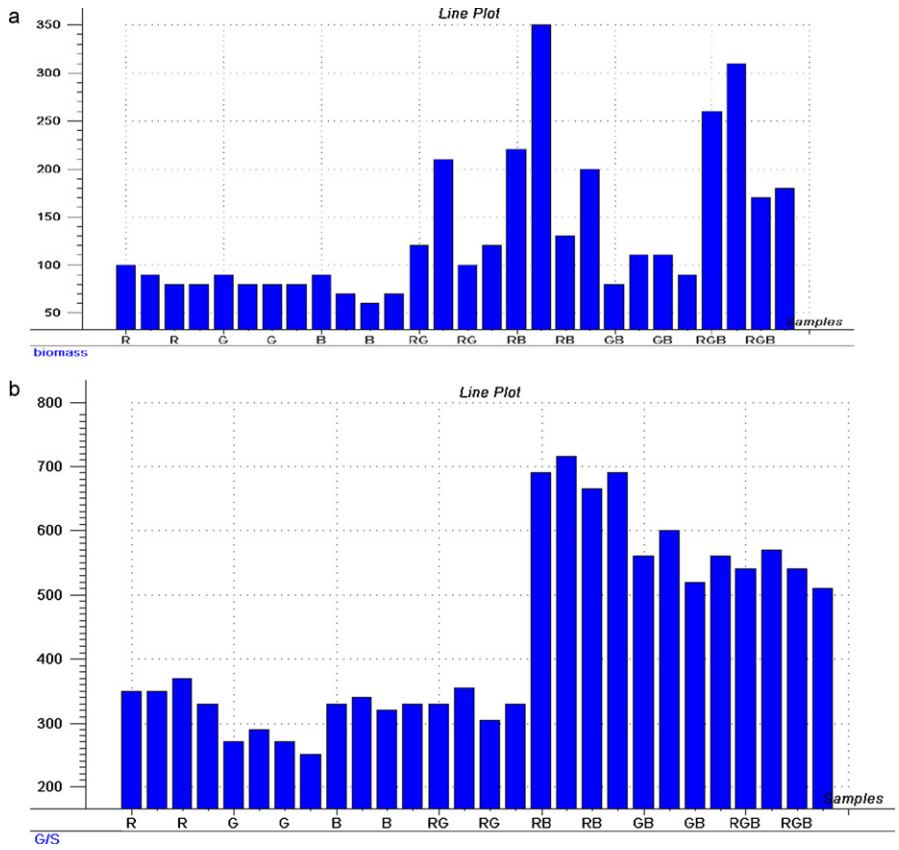

Fig. 6. Line plot variations: (a) biomass between grafted and the un-grafted samples; (b) gel strength obtained from κC samples of grafted and un-grafted strains.

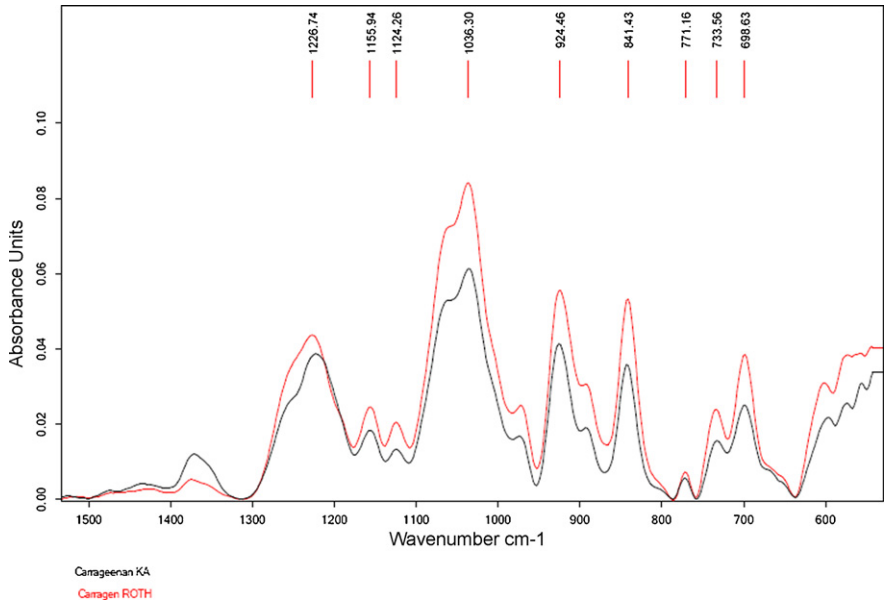

Fig. 7. The FTIR–ATR spectra of *kappa*-carrageenan, of *K. alvarezii* (obtained from RBG) and of commercially available *kappa*-carrageenan (M/s Carl Roth GmbH, Germany).

grams, genetic manipulation, somatic hybridization or transgenic production (Ask & Azanza, 2002; Cheney et al., 1994). The protocol reported in the prior art are very prolonged, complex, costly, and laborious, which required treatment of antibiotics, nutrient solution for isolation and callus culture to form the graft under *in vitro* conditions.

The result of this investigation indicates that the grafting protocol used in this study is very simple, easy to adopt and can be implemented directly *in vivo* conditions to improved the yields and quality of the biomass and product kC and supports our earlier study (Sahu, Ganesan, & Eswaran, 2010). The duration of attachment in the blends was depend on the colors of the strains, and was varied significantly. The physical attachment in the GB and RGB groups was observed after 21 and 25 days from the date of initial plantation. These results are in good agreement with our previous work (Sahu et al., 2010). Results of this study indicated that grafting of R + G and R + G + B strains produced superior quality grafted RG and RGB strains with greatest biomass yields, which also yielded greatest yield of kC with superior quality. This could be explained on the basis of their biocompatibility with each other as well as by the steadiness the essential environment conditions easily together. The DGR and biomass production were significantly greater in the grafted strains than those of the un-grafted R, B, and G strains. This result indicated that all the grafted strains prepared in this study could be utilized for improving the biomass of the commercially important seaweed *K. alvarezii* for production of commercially important phycocolloid *kappa*-carrageenan and liquid seaweed fertilizer (LSF). The similar observations have been reported in the literatures (Aguirre von Wobeser, Figuero, & Cabello-Pasini, 2001; Dawes et al., 1994; Hurtado, 1995; Munoz et al., 2004). No significant difference in the growth rates between different colored strains has been described by Trono and Ohno (1989). Studies on seasonal growth rate and carrageenan yields have been reported for the colored varieties of *K. alvarezii* and *E. denticulatum* (Azanza-Corrales & Sa-a, 1990; Dawes et al., 1994; Trono & Lluisma, 1992).

The seawater parameters analysis indicated presence of all the essential environmental parameters required for the growth of commercially important seaweed including *K. alvarezii*, and are in good agreement with those reported by Eswaran, Ghosh, and Mairh (2002). The enhanced growth of *K. alvarezii* could also be explained on these essential parameters, as concentrations of the essential components presence in the cultivation farm may be the favorable for the growth. According to Glenn and Doty (1990), temperature, light intensity and nutrients were believed to be the most important factors effecting the growth of *Kappaphycus* but these authors reported a low correlation between the growth and the environmental factors studied. Temperature of the sites has been considered as the main environmental factor affecting the growth rates of *Kappaphycus* (Glenn & Doty, 1990; Hurtado et al., 2001; Munoz et al., 2004; Ohno, Largo, & Ikumoto, 1994; Paula & Pereira, 2003; Trono & Ohno, 1989). Ohno et al. (1994) and Trono and Ohno (1989), have reported that plant grew well only during the warm weather season (20–30 °C). Glenn and Doty (1992) showed that photosynthetic response for *K. alvarezii* increased up to 32 °C, then sharply declined. The result of this study indicated that the temperatures of cultivation farm were in the range (22–31.5 °C), which needs for the proper growth of this seaweed. Further, the enhanced growth could also be explained on the basis of temperature factor, which was identical to that of reported for increased growth of *K. alvarezii* (Glenn & Doty, 1992).

Results of this study revealed that DGR and gelling properties of kC contents were improved significantly in the grafted strains (Tables 2–4). Sulphate content was decreased significantly when grafting R and G strains, while 3,6-AG content and gel strength were increased significantly (Table 3). These observations are in good agreements with our previous work (Meena et al., 2007). The decreasing pattern of sulphate and increasing trend of gel strength as well as 3,6-AG contents are in good combination to justify the results obtained in this study. The gel strength, which is the main measurement for the quality of carrageenan contents, was significantly greater in kC contents obtained from the grafted strains than those of un-grafted strains (Tables 3 and 4). This could be explained on the basis of increased 3,6-AG contents in kC products obtained from the grafted strains (Tables 3 and 4). In contrasting, a very low gel strength and gelling temperature kC product has been reported from the grafted strains of two species namely *Eucheuma cottonii* and *E. sipnosum* (Cheney et al., 1994). They have also concluded that the hybrid of *E. cottonii* and *E. sipnosum* can not be farmed in the tropics like *E. cottonii*. Results of this study indicated that this simple and cost effective grafting technique could be used for generating the superior quality biomass with improved yields, which

could also be used for preparation of superior quality kC products in greater yields. FTIR technique was used to confirmed the commercially important phycocolloid namely *kappa*-carrageenan from the above grafted and ungrafted strains (Pereira et al., 2009).

5. Conclusions

The results of the present study provides a simple, non-laborious and cost-effective protocol for vegetative propagation of *Kappaphycus alvarezii* and to improve the biomass and the quality of commercially important phycocolloid *kappa*-carrageenan, which are very useful for value addition of the red seaweeds and their products. Beside this, it is expected that the future research on this subject will develop an improved variety which not only help the phycocolloid industries but also can withstand the adverse environmental conditions like: grazing, high wave actions, high water current, changes in wind velocity, seawater temperature, etc. However, more experiments are needed in this subject to elucidate the above problems.

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References

Aguirre von Wobeser, E., Figuero, F., & Cabello-Pasini, A. (2001). Photosynthesis and growth of the red and green morphotypes of *Kappaphycus alvarezii* (Rhodophyta) from the Philippines. *Marine Biology*, *138*, 679–686.

Ask, E., & Azanza, V. (2002). Advances in cultivation of commercial eucheumatoid species: A review with suggestions for future research. *Aquaculture*, *206*, 257–277.

Ask, E., Batisbaga, A., Zertuche- Gonzalez, J. A., & de San, M. (2003). Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic location. In *Proceedings of international seaweed symposium, vol. 17* (pp. 49–57).

Azanza-Corrales, R., & Sa-a, P. (1990). The farmed *Eucheuma* species (Gigartinales, Rhodophyta) in Danajon reef, Philippines: Carrageenan properties. *Hydrobiologia, 204/205*, 521–525.

Bixler, H. J. (1996). Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia*, *326/327*, 35–37.

Cheney, D. P., Ipswitch, & Wang, L. Z. (1994). Methods of callusing somatic hybridization between two species of algae. U. S. Patents No. 5365018.

Craigie, J. S., & Leigh, C. (1978). Carrageenans and agars. In J. A. Hellebust, & J. S. Craigie (Eds.), *Handbook of phycological methods* (pp. 109–131). Cambridge: Cambridge University Press.

Dawes, C. J. (1992). Irradiance acclimation of the cultured Philippines seaweeds, *Kappaphycus alvarezii* and *Eucheuma denticulatum*. *Botanica Marina*, *35*, 189–195.

Dawes, C. J., Lluisma, A. O., & Trono, G. C. (1993). Clonal propagation of *Eucheuma denticulatum* and *Kappaphycus alvarezii* for Philippine farms. *Hydrobiologia*, *260/261*, 379–383.

Dawes, C. J., Lluisma, A. O., & Trono, G. C. (1994). Laboratory and field growth studies of commercial strains of *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the Philippines. *Journal of Applied Phycology*, *6*, 21–24.

Deslandes, E., Floc'h, J. Y., Bodeau-Bellion, C., Brault, D., & Braud, J. P. (1985). Evidence for i-carrageenans in *Solieria chordalis* (Solieriaceae) and *Calliblepharis jubata*, *Calliblepharis ciliate*, *Cystoclonium purpureum* (Rhodophyllidaceae). *Botanica Marina*, *28*, 317–318.

Deslandes, E., Potin, P., Zinoun, M., & Folc'h, J. Y. (1990). Contribution on the content and nature of the phycocolloid from *Kellymenia reniformis* (Cryptomeniales, Rhodophyta). *Hydrobiologia*, *204/205*, 603–608.

Doty, M. S. (1973). Farming the red seaweed, *Eucheuma*, for carrageenan. *Micronesica*, *9*, 59–73.

Doty, M. S. (1986). Estimating returns from producing *Gracilaria* and *Eucheuma* on line farms. *Monographic Biology*, *4*, 45–62.

Doty, M. S., & Alvarez, V. B. (1975). Status, problems, advances and economics of *Eucheuma* farms. *Journal of Marine Technological Society*, *9*, 30–35.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–366.

Eswaran, K., Ghosh, P. K., & Mairh, O. P. (2002). Experimental field cultivation of *Kappaphycus alvarezii* (Doty) Doty ex. P. Silva. at Mandapam region. *Seaweed Research Utilization*, *24*, 67–72.

Glenn, E. P., & Doty, M. S. (1990). Growth of seaweeds *Kappaphycus alvarezii, K. striatum* and *Eucheuma denticulatum*, as affected by environment in Hawaii. *Aquaculture*, *84*, 245–255.

Glenn, E. P., & Doty, M. S. (1992). Water motion affects the growth rates of *Kappaphycus alvarezii* and related seaweeds. *Aquaculture*, *108*, 233–246.

Hurtado, A. Q. (1995). Carrageeenan properties and proximate composition of three morphotypes of *Kappaphycus alvarezii* Doty (Gigartinales, Rhodophyta) grown at two depths. *Botanica Marina*, *38*, 215–219.

Hurtado, A. Q., Agbayani, R. F., Sanares, R., & Castro-Mallare, M. T. R. (2001). The seasonality and economic feasibility of cutivating *Kappaphycus alvarezii* in Panagatan Cays, Caluya, Antique Philippines. *Aquaculture*, *199*, 295–310.

Meena, R., Prasad, K., & Siddhanta, A. K. (2006). Synthesis of carrageenan-graft-PAAm: Evaluation of its adhesive and absorbent properties. *Journal of Applied Polymer Science*, *102*, 5144–5153.

Meena, R., Siddhanta, A. K., Prasad, K., Ramavat, B. K., Eswaran, K., Thiruppathi, S., Ganesan, M. A., Mantri, V., & Subba Rao, P. V. (2007). Preparation, characterization and benchmarking of agarose from *Gracilaria dura* of Indian waters. *Carbohydrate Polymers*, *69*, 179–188.

McHugh, D. J. (1987). Production, propoerties and uses of alginates. In: D.J. Mc Hugh (Ed.), *Production and utilization of products from seaweeds. FAO Fisheries Technical Report, 288*, 58-115.

McHugh, D. J. (2003). Seaweeds used as a source of carrageenan. In D. J. McHugh (Ed.), *A guide to the seaweed industry*. *FAO Fisheries Technical Report, 441*, 1–105.

Munoz, J., Freile-Pelegrin, Y., & Robledo, D. (2004). Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatan, Mexico. *Aquaculture*, *239*, 161–177.

Ohno, M., Largo, D. B., & Ikumoto, T. (1994). Growth rate, carrageenan yield and gel properties of cultured kappa-carrageenan producing red alga *Kappaphycus alvarezii* (Doty) Doty in the subtropical waters of Shikoku, Japan. *Journal of Applied Phycology*, *6*, 1–5.

Paula, E. J., & Pereira, R. T. L. (2003). Factors affecting growth rates of *Kappaphycus alvarezii* (Doty) Doty ex P. Silva (Rhodophyta, Solieraceae) in subtropical waters of Sao Paulo, Brazil. In *Proceedings of international seaweed symposium, vol. 17* (pp. 381–388).

Pereira, L., Amado, A. M., Critchley, A. T., van de Velde, F., & Ribeiro-Claro, P. J. A. (2009). Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). *Food Hydrocolloids*, *23*, 1903–1909.

Rincones, R. E. (2010). The introduction and cultivation of red alga *Kappaphycus alvarezii* for the production of carrageenan and the western Atlantic: An alternative livelihood for coastal communities. In *Proceedings of XX international seaweed symposium* (p. 93).

Robledo, D., & Freile-Pelrgrin, Y. (2010). Prespective for the cultivation of economic important carrageenophytes in southeast Mexico. In *Proceedings of XX international seaweed symposium* (pp. 94–95).

Sahu, N., Ganesan, M., & Eswaran, K. (2010). Inter- and intra-generic grafting in seaweeds in the Indian coasts. *Current Science*, *99*, 235–239.

Stanley, N. (1987). Production, properties and uses of carrageenan. In D. J. McHugh (Ed.), *Production and utilization of products from commercial seaweeds*. *FAO Fisheries Technical Paper, 288*, 116–146.

Strickland, J. D. H., & Parsons, T. R. (1972). *A practical handbook of seawater analysis*. Canada Bulletin: Fisheries Research Board., pp. 167-311.

Trono, G. C., & Ohno, M. (1989). Seasonality in the biomass production of *Eucheuma* strains in northern Bohol, Philippines. In I. Umezaki (Ed.), *Scientific survey of marine algae and their resources in the Philippine Islands* (pp. 71–80). Japan: Monbushio International Scientific Research Program.

Trono, G. C., & Lluisma, A. O. (1992). Differences in biomass production and carrageenan yields among four strains of farmed carrageenophytes in Northern Bohol, Philippines. *Hydrobiologia*, *247*, 223–227.

White, W., & Ohno, M. (1999). World seaweed utilization: An end of-century summary. *Journal of Applied Phycology*, *11*, 369–376.

Wolnik, K. A. (1988). Inductively coupled plasma-emission spectrometry. *Methods Enzymology*, *158*, 190–205.

Yaphe, W., & Arsenault, G. P. (1965). Improved resorcinol reagent for the determination of fructose, and 3,6-anhydrogalactose in polysaccharide. *Analytical Biochemistry*, *13*, 143–148.