Earlier studies on glaciers were based on the database and calibrated growth rates of the species to date the surfaces, which generally requires a long time period. The present technique is simple, concise and can generate a theoretical background for implementing future strategies for using lichens in climate change studies.


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Inter- and intra-generic grafting in seaweeds in the Indian coasts

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Traditionally, grafting has been effective among higher plants. An attempt was made to graft some commercially important red seaweeds in the shallow waters at Thonithurai, Mandapam on the south-east coast of India to study the possible effects of grafting on seaweeds. Lower group plants of Kappaphycus alvarezii (Ka), Gelidiella acerosa (Ga), Gracilaria verrucosa (Gv) and Gracilaria dura (Gd) were selected for intergeneric grafting. The graft combinations were Ka + Ga, Ka + Gv, Ka + Gd, Ga + Gv and Gv + Gd. Similarly, three different colour strains (green, red and conventional) of Ka were selected for intrageneric grafting. Four different combinations were made: R + C, R + G, G + C and R + G + C. Among intergeneric grafted plants, Ka + Ga was found attached after 29 days but could not survive after 60 days from the date of grafting whereas the plants of intrageneric grafts showed more than 80% survival rate. Grafts of R + C and G + C were attached after 3 weeks and R + G + C were attached after 25 days. There was good difference in their growth rate and the biomass yield. R + G + C gave an average yield of 443.3 g fr. wt plant−1. Analysis of variance (ANOVA) showed that intrageneric grafts between different strains of Ka had significant variations in their daily growth rate (F ratio = 1.20096E and P = 0.000). This study supports the feasibility of intrageneric grafting in seaweeds. Due to fast and easy regenerative potential, high grafting success is achievable.

Keywords: Intergeneric grafting, intrageneric grafting, polysaccharide, red seaweeds, regeneration.

The red seaweeds Kappaphycus alvarezii (Doty (Ka), Gelidiella acerosa (Forsskal) Feldmann et Hamel (Ga), Gracilaria verrucosa (Hudson) Papenf. (Gv) and Gracilaria dura (C. Agardh) J. Agardh (Gd) are in great demand for their cell wall polysaccharides. Because of fast growth rate, ease of reproduction and being an important source of k-carrageenan, the cultivation of Ka has obtained worldwide attention. The other seaweeds are significant sources of biomass for agar extraction in many countries[1–5]. The agar obtained from Ga and Gd is of superior quality and is widely used in a number of preparations in biomedical, biotechnological, pharmaceutical, food, cosmetic and paper industries[6–9]. The agar obtained from Gv is usually used in the food industry[10].

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The current world demand for agar and carrageenan is steadily increasing, placing a pressure on the presently overharvested natural sources and simultaneously increasing the importance of cultivation. In addition, the phycocolloid market lacks a stable supply of high quality raw material, emphasizing the need for selection and cultivation of improved varieties of seaweed species.

Cultivation conserves the natural resources and improves the elite germplasm. It is always desirable to make value additions in the existing germplasm through some easy methods which can be adapted easily by the cultivars. Cultivation technologies for important agarophytes like Ga and Ka have been well developed.

In order to develop an improved variety of seaweed with effective regeneration, grafting methods were attempted in Ka, Ga, Gv and Gd through experimental cultivation. Grafting is widely used for quick propagation of a desired crop as seen in rose. This is the first attempt towards the grafting of inter- and intra-generic seaweed species. The observations made in this study provide ample support to the amenability of grafting approach in seaweeds.

To study the feasibility of grafting in seaweeds, different commercially important red seaweeds from Thoni-thurai, Mandapam, south-east coast of India (09°17’N, 79°2’E) were used. The plants of Ga, Gv, Gd and different colour strains of Ka (red, green, brown-conventional variety) were collected from the Gulf of Mannar in May 2005. The fronds were washed thoroughly in seawater. Healthy fronds were selected for grafting. These fronds were cut obliquely to 8–10 cm length (Figure 1 a and b). Grafts were made by putting two straws together; a portion of thallus of the seaweed to be propagated was slipped on to the thallus of another, i.e. forming corresponding ‘mates’ and tied tightly (Figure 1 c and d).

The experiment was conducted in two different sets. In the first set, plants of Ka, Ga, Gv and Gd were selected for intergeneric grafting. In this set, the graft combinations were Ka + Ga, Ka + Gv, Ka + Gd, Ga + Gv and Gv + Gd. Similarly, in the second set of different strains of Ka were selected for intragenic grafting. Four different combinations were made: R + C, R + G, G + C and R + G + C. R, G and C represent the conventional variety, the red and green strains of Ka respectively. These combined plants were transferred to the farm for their attachment and further increasing their biomass through a modified protocol of cultivation.

The experiment was done by using bamboo rafts of 1.5 × 1.5 m. The length of the planting rope was 1 m. In each rope, 10 grafted plants and in each raft, five planting ropes were tied together.

Daily growth rate (DGR) of all sets was noted periodically, after 45 days from the date of planting.

DGR (%) was calculated using the formula:

\[ \text{DGR} = \frac{\ln(W_f/W_i) \times 100}{t} \]

where \( W_f \) is the final fresh weight (g) at \( t \) day, \( W_i \) the initial fresh weight (g) and \( t \) the number of culture days. Biomass yield \( Y \) (expressed as mean g fr. wt plant\(^{-1}\)) was determined using a modified formula to include the initial weight of the transplants, i.e.

\[ Y = \frac{(W_f - W_i)}{n} \]

where \( n \) is the number of grafted plants.

Samples of seawater were collected on monthly basis for analysing the hydrobiological parameters. Seawater temperature was recorded using standard centigrade thermometer. Salinity was measured using Atago Refractometer and pH by pH meter. PO\(_4\)-P, NO\(_3\)-N and NO\(_2\)-N were estimated using the standard methods.

The analysis of variance (ANOVA) was performed to study the comparison in the growth rate. It was done by using the software SYSTAT version 7.0.

This coast shows mixed substratum with mostly muddy bottom. The experimental area has a confluence of both Gulf of Mannar and Palk Bay waters.

Among the intergeneric grafts, plants of Ka and Ga (Ka + Ga) were found attached at their joints after 29 days from the date of planting (Figure 2). Grafts made with Gv, i.e. Gv + Ga, Gv + Gd and Ka + Gv were not attached at their joints. The percentage of survival was very less among the grafts of Ka + Ga. After 60 days, all grafts of Ka + Ga were found dead.

In another set, out of all combinations, plants of R + C and G + C were found attached at their joints after three weeks from the date of planting (Figure 3). Plants of R + G + C were found attached to each other after...
Table 1. Intragenic graft showing attachment days from the date of plantation, percentage of survival and their yield

<table>
<thead>
<tr>
<th>Combination</th>
<th>Conventional + red</th>
<th>Conventional + green</th>
<th>Red + green</th>
<th>Red + conventional + green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of grafts</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>No. of days</td>
<td>21</td>
<td>21</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Percentage of survival</td>
<td>82</td>
<td>86.67</td>
<td>85.3</td>
<td>93.33</td>
</tr>
<tr>
<td>Biomass (Y = g fr. wt/plant), n = 50</td>
<td>281.1</td>
<td>156.46</td>
<td>316.4</td>
<td>443.3</td>
</tr>
<tr>
<td>Biomass (Y = kg wt/raft)</td>
<td>14.055</td>
<td>7.823</td>
<td>15.820</td>
<td>22.165</td>
</tr>
</tbody>
</table>

Table 2. F-ratio and p-value showing variations in daily growth rate

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-squares</th>
<th>d/</th>
<th>Mean-square</th>
<th>F-ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
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<td>3</td>
<td>6.667</td>
<td>1.20096E</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>0.000</td>
<td>12</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post hoc test of DGR

Tukey HSD multiple comparisons

Matrix of pairwise comparison probabilities

<table>
<thead>
<tr>
<th></th>
<th>RG</th>
<th>RC</th>
<th>GC</th>
<th>RGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RC</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>RGC</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*p < 0.001.

Values with significance level are marked in bold.

Figure 2. Intergeneric grafting showing attached portion (after 29 days) between *Kappaphycus alvarezi* and *Gelidiella acerosa*. *Arrows* showing the grafting zone.

25 days from the date of planting (Table 1). Around 80% of all grafts were found growing well. A good difference was observed in the biomass yield per plant as well as per raft (Table 1). R + G + C was found with an average yield per plant of 443.3 g fr. wt followed by R + G (316.4 g fr. wt), R + C (281.1 g fr. wt) and G + C (156.46 g fr. wt) respectively.

Seawater temperature ranged between 24°C and 32°C and the salinity was found between 28 and 34 ppt during the study period. Dissolved oxygen and pH content of seawater were recorded within the range of 0.8–1.6 mg l⁻¹ and 7.7–8.2 mg l⁻¹ respectively. Nitrite, nitrate and phosphate content of seawater were recorded between 1.2 and 6.8 μM, 0.008 and 0.45 μM, and 0.38 and 6.46 μM respectively throughout the study period.

ANOVA showed that DGR among the intragenic grafts, i.e. combinations made between different strains of *Kappaphycus* had significant variations (F-ratio = 1.20096E and p = 0.000). All grafts varied significantly with each other (p = 0.000), i.e. RG showed variations with RC, GC and RGC. Similarly, RC varied significantly with GC and RGC (Table 2).

Our study suggests that the coast with the required environmental factors favours the growth of the grafted plants. The seawater temperature ranging between 25°C and 30°C and salinity between 30 and 34 ppt is best for growing *Eucheuma*22,23. The nitrogen and phosphorus range supports the previous study made on the cultivation of *Kappaphycus* and *Ga*11,14,24.

The present study suggests that the chances of producing intragenic grafts are favourable in a vegetative propagation system especially in red seaweeds, where the cell wall is usually rich in mucilage. It is presumed that the polysaccharides present in their thalli help in attaching the corresponding mates. This study provides ample support to the feasibility of grafting in seaweeds. However, the result recorded among the intergeneric grafts does not support this. This may be because of their different anatomical, physiological and chemical properties. The success of grafting process in different strains of *Kappaphycus* could be because of fast and easy reproductive potential. The study shows significant variation in the growth rate and high biomass in the grafted plants with a combination of all three strains (R + C + G). Laboratory and field studies show slightly higher growth rates for the green strain when compared to brown or red25,26. There was no
significant difference observed in the growth rates between the brown and green colour strains cultured in northern Bohol. Recently, it has been shown that the differences in the pigment content for these cultivars had no effect on either photosynthesis or growth.

The present study provides a simple and easy system for vegetative propagation in red seaweeds. Because seaweeds have enormous regenerative potential, it can be said that with a bit of patience, high grafting success is achievable.

It is expected that future research on this subject may help the phycocolloid industries by producing seaweeds with good quality and high yield of phycocolloid contents. Besides, significant improvements in seaweed strains can be achieved through grafting interventions which can withstand the adverse environmental conditions like grazing, high wave actions, high water current, changes in wind velocity, seawater temperature, etc. However, further experiments are needed to elucidate the chemical and physiological responses of these and other different types of seaweeds.

Errata

Anomalous cooling over the Arabian Sea during February 2008

Page 1616, para 1: ‘Air temperature over the land was higher by 6–8°C with respect to air temperature over the ocean’.

should read as

‘Air temperature over the land was lower by 6–8°C with respect to air temperature over the ocean.’

I regret the error.

– Anant Parekh

P. C. Vaidya (1918–2010)
[Jayant V. Narlikar, Curr. Sci., 2010, 98, 1389]

Column 1, line 3:
‘died on 8 April 2010 ...’ should read as
‘died on 12 March 2010 ...’

Column 2, line 5:
‘... Vallabhshri Patel College...’ should read as
‘... Vithalbhai Patel College...’

I regret the errors.

– Jayant V. Narlikar