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Healing and regeneration responses in *Gigartina skottsbergii* (Rhodophyta, Gigartinales): optimization of vegetative propagation for cultivation

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Abstract

The red alga *Gigartina skottsbergii* is becoming increasingly valuable as a resource to providing the raw material for the carrageenan industry established in Chile and elsewhere. As a result, wild stocks of the species are subject to intense harvesting by local fishermen. With the current levels of harvesting, it seems likely that natural stands of *G. skottsbergii* will soon collapse. Although cultivation seems an alternative, knowledge regarding the biology of the species is exceedingly limited. This study reports the first attempt to determine the optimal conditions for vegetative propagation of this species in the laboratory. For this purpose, the processes of wound healing and regeneration of frond fragments and haptera were studied under controlled conditions of temperature, light, and media strength. Our results demonstrated that excised tissues of *G. skottsbergii* were able to seal the exposed areas in approximately 20 days, by a wound healing process characterized by a re-differentiation of medullary cells into a normal cortex. Our data also demonstrated that frond fragments are better than haptera for propagation purposes. The development of new cortical tissue at the cut surface is followed, within 60 days, by localized blade-like outgrowths along the repaired area. Furthermore, the healing and regenerative responses in both frond fragments and haptera differed in efficiency according to the various combinations of factors, with optimum of 10–15 °C, 5 μmol m^{-2} s^{-1} and plain seawater or standard SFC medium for the fronds. The two types of responses were negatively affected by seawater enriched with a double concentration of nutrients.

Introduction

The rhodophycean *Gigartina skottsbergii* is endemic to southern South America, and has been reported on the Pacific coast from Valdivia to Cape Horn and on the Atlantic coast in the Falkland Islands (Santelices, 1989). This is a subtidal species, harvested by local fishermen, and in recent years has become one of the most important algal resources in Chile. Together with *Sarcothalia* and *Mazzaella*, *G. skottsbergii* constitutes the basis for the carrageenan industry, which has established several processing plants to extract the colloid in the country (Buschmann et al., 1999).

Considering the sustenance of the resource, it is becoming increasingly apparent that the present harvesting rate of 4000 t yr^{-1} dry weight imposes a severe pressure on the wild stocks of this alga, and the available information indicates that some stands have already been abandoned by fishermen as a result of over-exploitation. Even though the experience of over-exploitation of an algal resource is not new in Chile (i.e. *Gracilaria*), the situation with *G. skottsbergii* is qualitatively different. With *Gracilaria*, the understanding of its biology facilitated the development of farming operations, which have almost replaced the biomass originally harvested from wild

stocks (Buschmann et al., 1995, 1999). In *G. skottsbergii*, on the other hand, the information is scarce and focused on taxonomy (Kim, 1976), phenology and standing stock assessment (Zamorano & Westermeier, 1996; Piriz 1996), and chemical composition (Palermo et al., 1984; Cerezo, 1986; Noseda, 1989; Matulewics et al., 1990; Piriz & Cerezo, 1991; Schnettler et al., 1995). Thus, the lack of information on the ecology, physiology, reproductive biology, among other aspects, precludes the development of cultivation techniques aimed at alleviating the negative impact of indiscriminate harvesting currently affecting wild stocks of *G. skottsbergii* (Buschmann et al., 1999).

As part of a long-term research program designed to obtain basic biological information on *Gigartina skottsbergii*, we have concentrated on two aspects considered of primary importance to setting the basis for its cultivation. First, we are optimizing the production of juveniles from spores under laboratory conditions, to provide seedlings for re-planting overexploited areas or to supply eventual farmers with adequate inoculum (unpublished). Our observations from wild populations of *G. skottsbergii* have revealed, however, that viable spores are available only for a very short period of time. Therefore, the second aspect needing to be studied is the development of alternative methods of obtaining new plants, namely through vegetative propagation (i.e. thallus fragmentation, tissue culture or protoplasts regeneration). Considering economical and practical reasons, our first choice was to assess the feasibility of vegetative propagation of *G. skottsbergii* through thallus fragmentation.

In algae belonging to the genus *Chondrus* (Chen & Taylor, 1978) *Gracilaria* (Goldstein, 1973; Santelices & Varela, 1995) and *Eucheuma* (Doty, 1987) the process of vegetative propagation through thallus fragmentation is well known and it has allowed a rapid expansion in farming of these species in different parts of the world. A successful vegetative propagation involves two closely related processes: wound healing and regeneration of new thalli. Whereas the first process is critical to prevent bacterial infections and subsequent thallus degradation, the second process is the key to re-establishing the normal growth of the alga. Studies on healing and regeneration are available for various species of red algae, including *Eucheuma alvarezii* var. *tambalang* Doty (Azanza-Corrales & Dawes, 1989), *Schottera nicaeensis* (Lamouroux et Duby) Guiry et Hollemberg (as *Petroglossum nicaeense*) (Perrone & Felicini, 1972), *Gigartina acicularis* (Wulf.) Lamouroux (Perrone & Felicini, 1976), *G. exasperata* Harvey et Bailey (Sylvester & Waaland, 1983) and *Grateloupia acuminata* Okamura (Iima et al., 1995). In all these cases, the processes of healing and regeneration seem to be easily achieved in the laboratory, suggesting that vegetative propagation is a good method of generating propagules for large scale cultivation. However, there is an exceedingly limited amount of information regarding the regulatory effect of physical factors such as temperature, light, etc., on both processes. This is important because our preliminary trials showed that fragments of *G. skottsbergii* have high tendency to undergo rapid tissue degradation in the laboratory, particularly when fragments are small (0.25 cm^2 or less). Those results stressed the need to understand the factors regulating the processes of wound healing and regeneration, before tissue cuttings can be used as an alternative for large scale propagation of *G. skottsbergii*. Therefore, in this study we characterized the healing and regeneration responses in excised fragments of fronds and haptera of *G. skottsbergii*, and assessed the regulatory role of temperature, light, and the quality of the culture medium on these two processes.

Materials and Methods

Immature fronds of *Gigartina skottsbergii* were collected by SCUBA diving, from a population occurring at 10–15 m deep in Ancud Bay (41°48′ S, 73°50′ W), and transported overnight to the laboratory in Santiago, 1000 km north of the collecting site, in a container at 4 °C. Immediately after their arrival, fronds were put in freshly filtered seawater from the collecting site (at 10 °C), where they remained during the manipulations prior to the experiment. Each frond was carefully brushed and rinsed in running tap water followed by rinses in filtered seawater from Ancud. A total of 50 fronds, ca. 900 cm^2 each, were used to obtain 810 excised fragments of 4 cm^2 from the center and 810 from the border areas. Fragments from each pool (i.e. border and center area) were then randomly assigned to each of the experimental treatments. Each experimental treatment included three 500-mL Erlenmeyer flasks with 400 mL of the appropriate medium, and each flask was inoculated with 10 of the randomly selected fragments. The experimental design included photon flux densities of 5, 25 and 50 μmol m^{-2} s^{-1} temperatures of 5, 10 and 15 °C, and culture media consisting of plain filtered seawater from the collecting site, standard SFC (Correa et al., 1988) and

2 × SFC (only the macronutrients were doubled in the latter culture medium). Flasks were thoroughly agitated twice a day and the culture medium was changed twice a week. The photoperiod was kept constant at 16:8 (L:D) and observations of the changes in the tissues at the cut edge were monitored once a week. Wound healing was recorded as completed when the exposed tissues at the wound showed a fully recovered deep purple pigmentation, typical of healthy cortical tissues. These observations were done with a stereo microscope Nikon Optiphot 2 using sections of unstained frozen tissue, perpendicular to the repaired surface.

The same material and experimental design used during the first stage (healing), was used in the second stage (regeneration), and the timing of the observations was as in the first set of experiments. Positive regeneration was recorded when a healed fragment issued one or more blade-like outgrowths from the repaired area.

The experiments with haptera followed the same protocol used with the fragments of fronds, in both wound healing and regeneration experiments. Haptera, 5mm long, were excised from plants collected in Ancud and incubated in 50 mL flasks filled with 45 mL of the respective culture medium (see above).

Percent data were arcsine transformed and analyzed by a 3-way ANOVA using SYSTAT (Wilkinson et al., 1992).

Results

The frond of *Gigartina skottsbergii* is more or less circular in profile (Figure 1a), 1–3 mm thick, leathery, and at least part of the surface of cystocarpic individuals is usually covered with papillae. In a cross section (Figure 1b), the thallus consists of 3 distinctive regions. Externally is the deeply pigmented outer cortex, 5–6 cells thick, which is covered by a thick and refringent outer cell wall. Cells in this layer are spheroid, 4–6 μm in diameter, and surrounded by a relatively thin cell wall. Immediately underneath the outer cortex is a poorly defined inner cortex of 2–3 layers of irregularly shaped and pigmented cells, 8–10 μm in diameter, which show multiple connections with neighbor cells. Finally is the medulla, which consists of cylindrical, elongated and profusely branched cells, 4–6 μm in diameter and in cases, longer than 100 μm. These thin medullar cells have thick cell walls, and present numerous anastomosis with neighbor cells, which results in a medulla with a 3-dimensional network appearance.

Very minor changes were observed during the first few days after the fragments were excised from the fronds. Only the cells cut by the razor blade lost the cytoplasmic content, an effect noticed mainly in medullar cells due to their size and shape. Also apparent during the first week was the presence of a mucilaginous plug at the cut, which dissolved as the process of healing progressed. At day 7 after excision, a clear cell differentiation process was noticed, which was characterized by the formation of short filaments directly from the subcortical and medullar cells at the cut (Figure 1c). Cortical and medullar cells located immediately beneath the cut, however, remained unaltered. The newly formed filaments were short, containing 2–3 club-shaped to more or less isodiametric cells, deeply pigmented and covered by thick cell walls. This phenomenon extended quickly throughout the exposed tissues, including the cortical region of the cut. It is important to mention that under sub-optimal conditions, wound healing takes place at 0.5–1 mm below the actual cut surface, which is observed macroscopically as a thin layer of whitish and softened tissue. This whitish tissue is eventually sloughed off the wounded areas. Continuous cell proliferation at the cut resulted in a gradual repair of the wound, which at day 14 after excision was characterized by a clearly enhanced cell density (Figure 1d). Although not yet fully developed at this stage, the typical thick outer cell wall normally found external to the cortex throughout the frond begins to become apparent, usually associated with the original cortex at the cut (Figure 1d). About 20 days after excision (Figure 1e), the first 1–2 layers of truly outer cortical cells became well defined, and a continuum was established between this new layer and the original cortex, located at the borders of the cut. At this point, a more advanced stage of development of the normal outer cell wall, up to 25 μm thick and translucent, was also observed (Figure 1e). Finally, at day 30 (Figure 1f) the normal cortical tissue developed fully at the wound. This new cortex consisted of up to 10 layers of small, almost isodiametric cells, 4–6 μm in diameter, which completely sealed the wound. Once the above process of healing concluded, localized regenerative areas appeared along the sealed borders of the fragments, which resulted in the production of multiple blade initials (Figure 1g). More developed stages of these blade-like outgrowths (Figure 1h) are commonly found in the field.

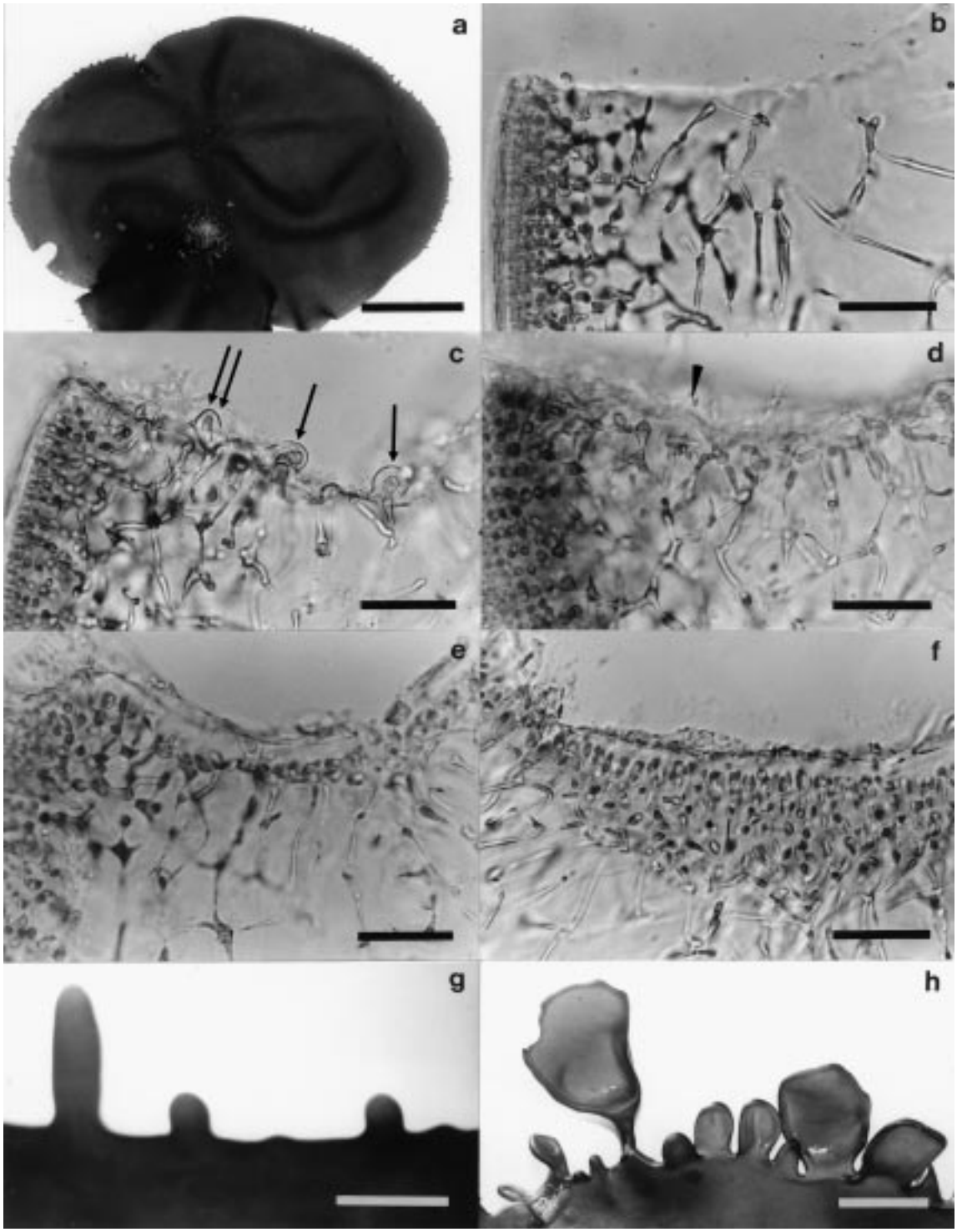

Figure 1. *Gigartina skottsbergii* a) Wild immature frond from Ancud. Scale: 5 cm. b–f) Cross-section through the wound at various stages of healing b) Freshly cut fragment, at the beginning of the healing experiment. Scale: 50 μm. c) Day 7, with short filaments emerging from the subcortical (single arrows) and medullar (double arrow) cells. Scale: 50 μm. d) Day 14, with increased cell density at the surface of the cut and the early development of the outer cell wall (arrowhead). Scale: 50 μm. e) Day 20, the cortex begins to cover the surface of the wound. Continuity between this new layer and the original cortex is observed on the left side of the section. The protective translucent outer cell wall is well developed and covers the new cortical tissue. Scale: 50 μm. f) Day 30, a fully developed cortex is established, which seals the wound. Scale: 50 μm. g) Regeneration at the edge of the fragments. Uprights growing directly from the new cortical tissue developed at the wound. Scale: 2 mm. h) A more advanced regeneration stage in a wild frond from Ancud. Scale: 2 cm.

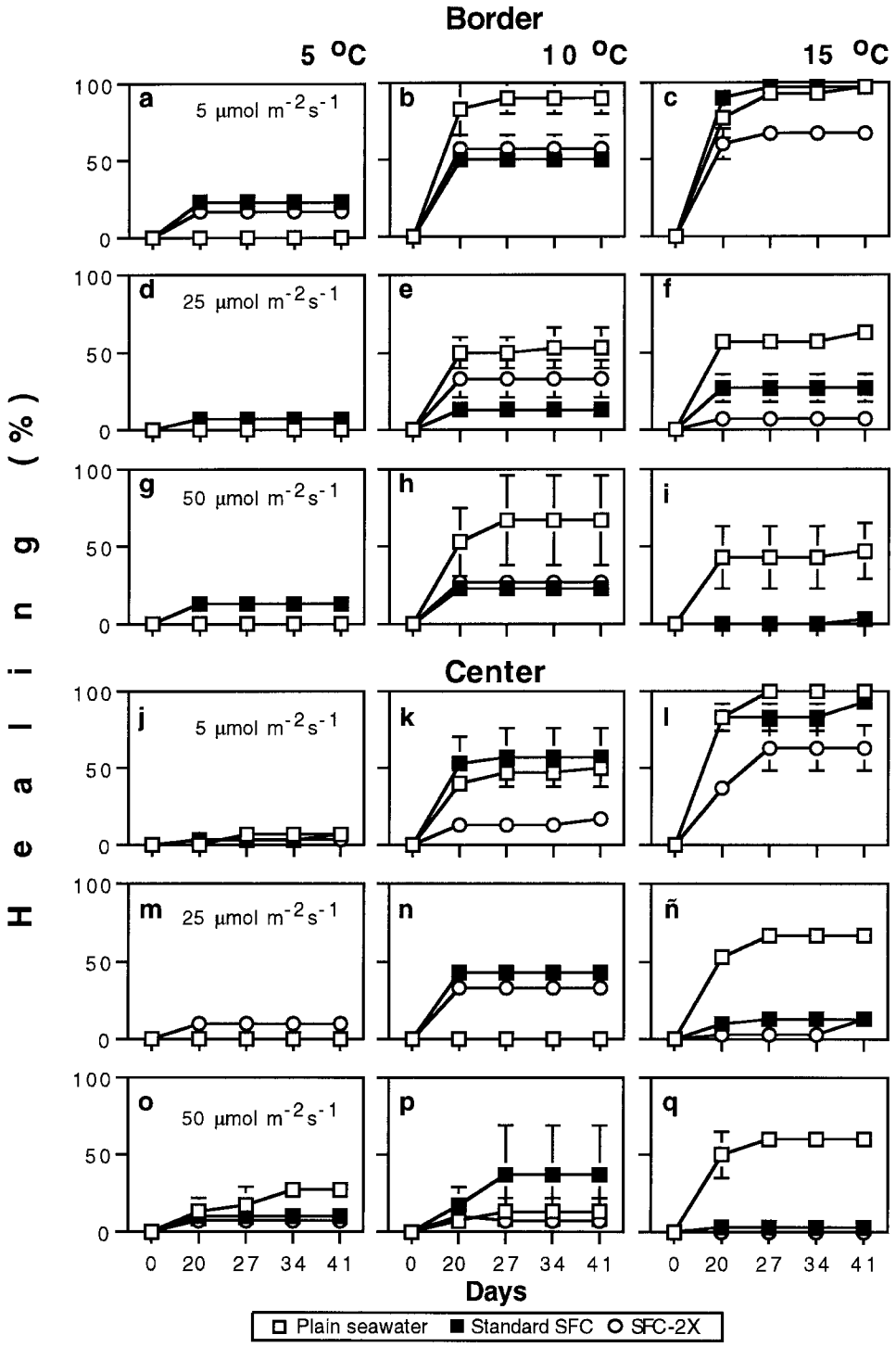

Figure 2. Healing of *Gigartina skottsbergii* tissue excised from the border and the center of fronds from Ancud, incubated under combined regimes of temperature, photon flux density and medium strength. Values represent mean and standard error.

Table 1. Summary of ANOVA with the effects of temperature, photon flux density and strength of medium on wound healing in tissues obtained from the border and center of *Gigartina skottsbergii* fronds.

Treatment	Border			Center		
	d.f	F-value	Probability	d.f.	F-value	Probability
Temperature (T)	2	61.5	$P \ll 0.001$	2	27.8	$P \ll 0.001$
Photon fluence rate (PFR)	2	43.7	$P \ll 0.001$	2	23.8	$P \ll 0.001$
Medium concentration (MS)	2	12.1	$P < 0.001$	2	4.75	$P < 0.02$
T × PFR	4	9.44	$P < 0.001$	4	11.8	$P \ll 0.001$
T × MS	4	14.6	$P \ll 0.001$	4	12.0	$P \ll 0.001$
PFR × MS	4	1.01	$P > 0.41$	4	3.12	$P < 0.03$
T × PFR × MS	8	1.27	$P > 0.28$	8	1.75	$P > 0.11$

The healing responses of frond fragments of *Gigartina skottsbergii* were significantly influenced by temperature, light and media strength (Table 1, Figure 2). Furthermore, significant interactions were detected between temperature and light, and between temperature and medium strength (Table 1). This pattern of response was similar in fragments of tissue obtained from the border (Figures 2a–i) and from the center (Figures 2j–q) of the fronds. The strongest effect appeared to be induced by temperature, which at 5 °C and regardless photon flux density, medium strength, and the type of tissue, did not allow the fragments to reach high levels of healing (Figures 2a, d, g, j, m, o). On the other hand, at 10 and 15 °C and particularly in tissues from the border, significant increases in the percentage of healing success were recorded, varying according to the combined effects of light intensity and medium strength. Under these temperatures, mean values of fragments fully healed higher than 40% were achieved by using plain seawater, reaching almost 100% when photon flux density was 5 μmol m^{-2} s^{-1}. In tissues from the center of the frond, however, healing success near 100% was recorded only at 15 °C, with either plain seawater or standard SFC, and 5 μmol m^{-2} s^{-1} (Figure 2–l). Thus, a clear qualitative trend emerges from the above experiments, indicating that the best combination of factors to achieve high rates of successful healing is 10-15 °C, 5 μmol m^{-2} s^{-1} and plain seawater or standard SFC medium (Figure 2). Interestingly, increasing the concentration of nutrients resulted in a deleterious effect on the healing in *G. skottsbergii*, a trend particularly noticeable at higher temperatures. Finally, and regardless of the experimental treatment, the healing process was completed 20–27 days after the excision. Those fragments which had not healed during that period began to bleach and eventually degraded.

Those fragments which had completed the healing process were kept under the same culture conditions, and their capacity to regenerate by issuing new shoots is summarized in Figure 3. Temperature, photon flux density and medium strength, all had significant effects on the production of new shoots, regardless the origin of the fragments, i.e. border or center of the frond (Table 2). All interactions were also significant, indicating a synergistic effect of the combined factors (Table 2). Regeneration efficiency was higher in fragments excised from the border (Figures 3a–i) and from the center (Figures 3j–q) when combining higher temperature (15 °C), lower photon flux density (5 μmol m^{-2} s^{-1}) and plain seawater. In a time context, 55–62 days were needed to complete the regeneration process, with fragments issuing numerous shoots from the cut edges (Figure 1g). By using the optimum combination outlined above, 60 and 80% of the fragments from the border and center respectively can be induced to produce new fronds (Figure 3). Although the regenerated cuttings incubated in plain seawater developed a greenish pigmentation at the end of the experiments, a 1-week incubation in standard SFC restored the normal pigmentation of these fragments.

Haptera are cylindrical, finger-like projections of vegetative tissue which are issued in large numbers by both sporophytic and gametophytic fronds of *Gigartina skottsbergii*, from the side facing the substratum (Figure 4a). The wound healing and regeneration processes of haptera are almost identical to those observed in frond fragments of *G. skottsbergii* (Figure 4b–f). The time needed for the initial formation of

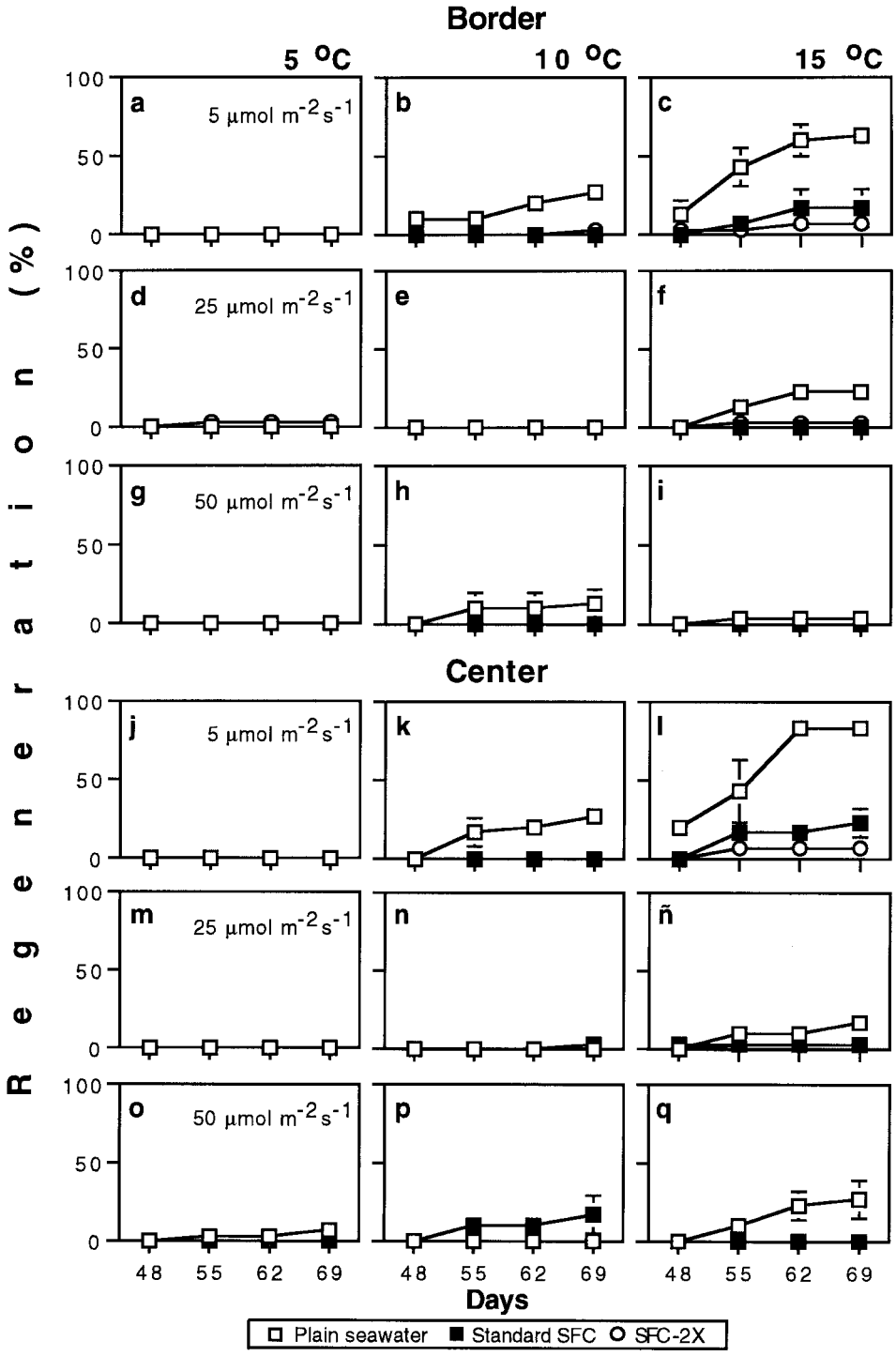

Figure 3. Regeneration of *Gigartina skottsbergii* tissue excised from the border and the center of fronds from Ancud, incubated under combined regimes of temperature, photon flux density and medium strength. Values represent mean and standard error.

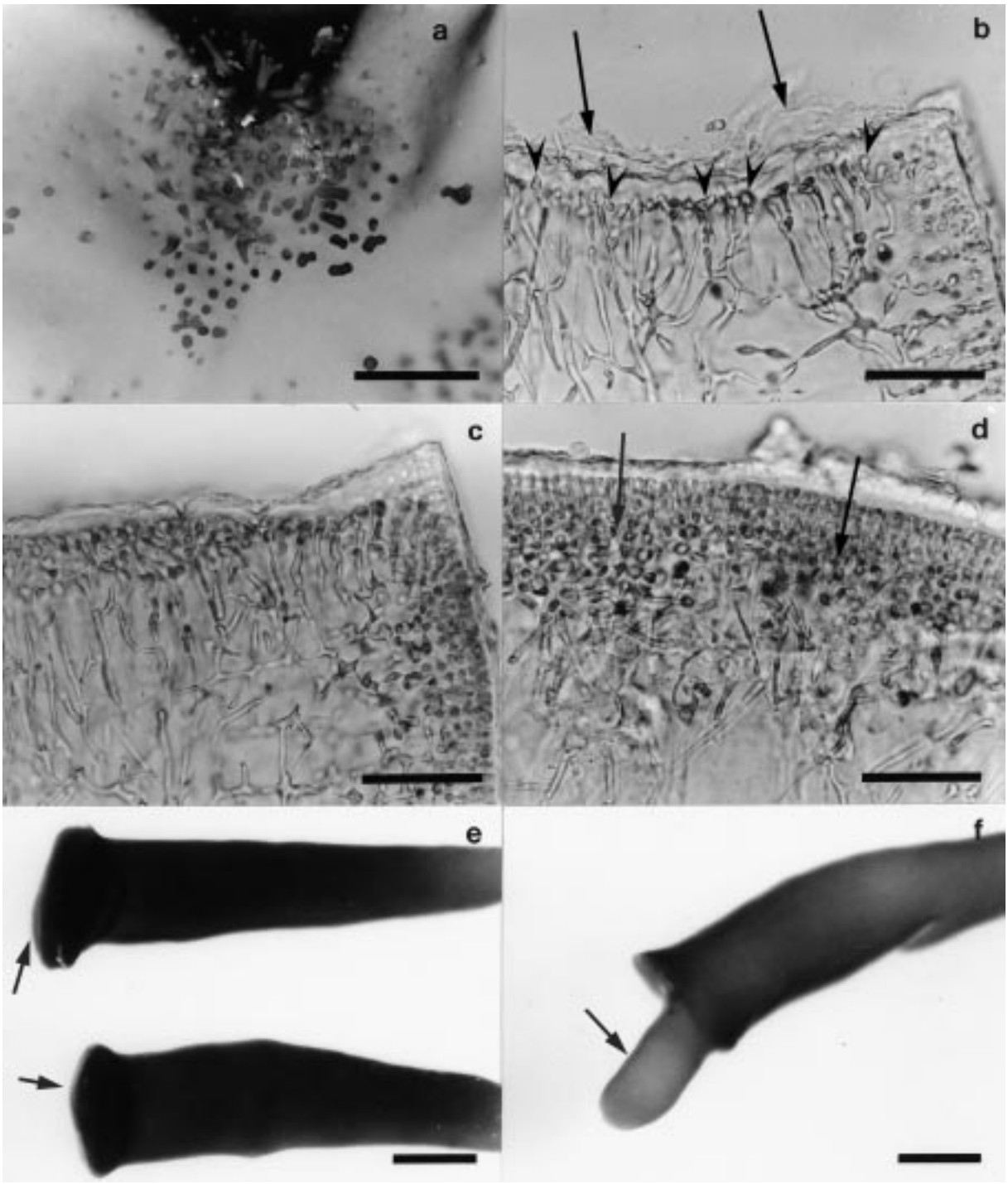

Figure 4. Healing and regeneration in haptera of *Gigartina skottsbergii*. a) Numerous haptera on the surface of a cystocarpic frond. Scale: 15 mm. b–d) Cellular changes at the cut surface: b) At day 14, with a single layer of small, more or less isodiametric cells (arrowheads) covering the wound. A translucent outer cell wall is well developed (arrows) and is external to the young cortex. Scale: 50 μm. c) Day 21, up to four cell layers are forming the new cortex at the wound. The connection with the old cortex, on the right side of the section, is apparent. Scale: 50 μm. d) Day 60, a fully developed cortex (arrows) has developed, sealing the cut. Scale: 50 μm. e) Healed haptera with a flat, dark scar (arrows) covering the original wound. Scale: 1 mm. f) Regenerated haptera with a single upright (arrow) emerging directly from the new cortex. Scale: 1 mm.

Table 2. Summary of ANOVA showing the effects of temperature, photon flux density and strength of medium on regeneration in tissues obtained from the border and center of *Gigartina skottsbergii* fronds.

Treatment	Border			Center		
	d.f	F-value	Probability	d.f.	F-value	Probability
Temperature (T)	2	22.4	$P \ll 0.001$	2	36.9	$P \ll 0.001$
Photon fluence rate (PFR)	2	18.5	$P \ll 0.001$	2	13.3	$P \ll 0.001$
Medium concentration (MS)	2	22.7	$P \ll 0.001$	2	30.0	$P \ll 0.001$
T × PFR	4	8.79	$P < 0.001$	4	8.50	$P \ll 0.001$
T × MS	4	8.07	$P < 0.001$	4	13.0	$P \ll 0.001$
PFR × MS	4	4.53	$P < 0.004$	4	6.55	$P < 0.001$
T × PFR × MS	8	2.34	$P < 0.031$	8	4.15	$P < 0.001$

Table 3. Summary of ANOVA with the effects of temperature, photon flux density and strength of medium on wound healing in haptera obtained from *Gigartina skottsbergii*.

Treatment	d.f	F-value	Probability
Temperature (T)	2	16.53	$P \ll 0.001$
Photon fluence rate (PFR)	2	0.98	$P = 0.381$
Medium concentration (MS)	2	3.57	$P < 0.035$
T × PFR	4	0.59	$P = 0.671$
T × MS	4	5.66	$P < 0.001$
PFR × MS	4	1.20	$P = 0.321$
T × PFR × MS	8	0.85	$P = 0.557$

Table 4. Summary of ANOVA, showing the effects of temperature, photon flux density and strength of medium on regeneration responses of haptera obtained from *Gigartina skottsbergii* fronds.

Treatment	d.f	F-value	Probability
Temperature (T)	2	35.87	$P \ll 0.001$
Photon fluence rate (PFR)	2	12.60	$P < 0.001$
Medium concentration (MS)	2	0.46	$P = 0.631$
T × PFR	4	7.03	$P < 0.001$
T × MS	4	1.39	$P = 0.247$
PFR × MS	4	3.18	$P < 0.020$
T × PFR × MS	8	1.53	$P = 0.168$

the outer cell wall and the single-layer cortex (4b), structures which sealed the wound, as well as the time required for the establishment of the 3–4 cell layer cortex (Figure 4c) was 14 and 21 days respectively. The final thick cortex developed after 2 months in culture (Figure 4d), the time at which macroscopically a flat and dark scar was observed at the wound (Figure 4c). Within a month after the wound was healed, upright axes were issued from the scar (Figure 4f).

Healing success in haptera was significantly affected by temperature and media strength as well as by the interaction between these two factors (Table 3). The general trend shows (Figure 5a–i) that a consistently higher success in healing was achieved at 10 °C, with minor differences due to media strength and none to photon flux density (Figure 5b, e, h). Lower success was recorded at 5 °C, where healing values fluctuated between 30 and 70% (Figure 5a, d, g). At 15 °C, on the other hand, the responses were strongly influenced by media strength, with plain seawater being the best culture medium, triggering almost 100% of healing success (Figure 5c).

The production of uprights from the healed region of the haptera was significantly influenced by temperature and photon flux density, as well as by the interaction between the later factor and temperature and media strength (Table 4). Overall, however, the production of new uprights from haptera was poor (Figure 6a–i). In the 2-mo. experimental period, the best results were obtained at 10 °C (Figure 6b, e, h), although in no case was more than 50% success achieved. In most abiotic combinations the production was exceedingly low (Figure 6a, c, d, g) or nil (Figure 5f, i). In spite of the above, haptera remained healthy throughout the experimental period, and in most cases elongation was observed.

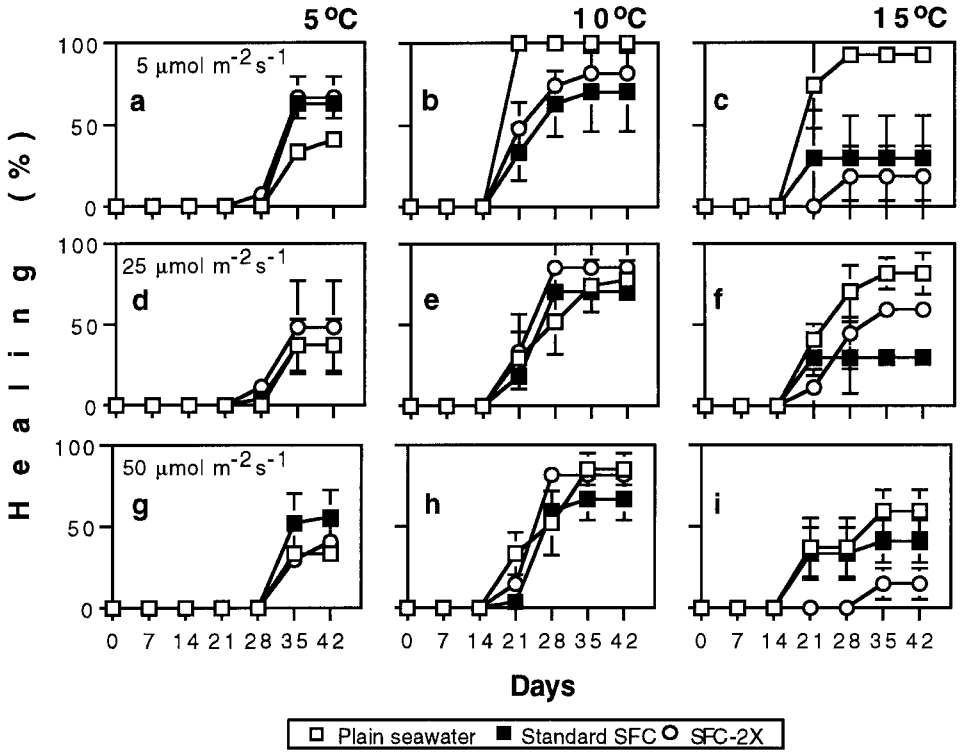

Figure 5. Healing of *Gigartina skottsbergii* haptera from Ancud, incubated under combined regimes of temperature, photon flux density and medium strength. Values represent mean and standard error.

Discussion

Our study demonstrated that wound healing and regeneration of *Gigartina skottsbergii* can be controlled under laboratory conditions, opening the possibility of using vegetative propagation as an alternative method of producing propagules at large scale. At the same time, this study strongly suggests that the best alternative is to produce those propagules from frond fragments rather than from haptera. This conclusion is based on the fact that, even though wound healing of haptera can be reasonably regulated in the laboratory, the production of frond initials is significantly lower than in frond cuttings. It is clear now, with frond cuttings, that a proper combination of photon flux density, temperature and the type of culture medium (i.e. 5 μmol m^{-2} s^{-1}, 15 °C, plain seawater) may improve healing rates to values of up to 100%. Also important is that cuttings from the center of the frond healed as well as those from the border. This finding is relevant from a management point of view, where there is a need to optimize the yield of useful cuttings from adult stock plants. Similarly, regeneration is optimized by applying the same combination of factors established to obtain the best healing results. In the case of regeneration, the values (60 and 80% of fragments from the border and center respectively) are certainly underestimated because of the decision to end the experiment at day 69. In fact, our experience indicates that every fragment which successfully completes the process of healing, eventually issues new shoots from the newly produced cortical tissue at the sealed wound.

Comparison of our results in a broader context, however, is difficult because almost all the existing evidence regarding healing and regeneration in other algae concentrates in a qualitative description of the phenomena. Thus, even though the cellular and tissue events taking place during healing and regeneration are known for several species of foliose Rhodophyta (Perrone & Felicini, 1972; 1976; Sylvester & Waaland, 1983; Azanza-Corrales & Dawes, 1989; Iima et al., 1995) and Phaeophyta (Fulcher & McCully, 1969; 1971; Fagerberg & Dawes, 1976), information regarding the potential regulatory effects exerted by abiotic factors on those processes is limited to

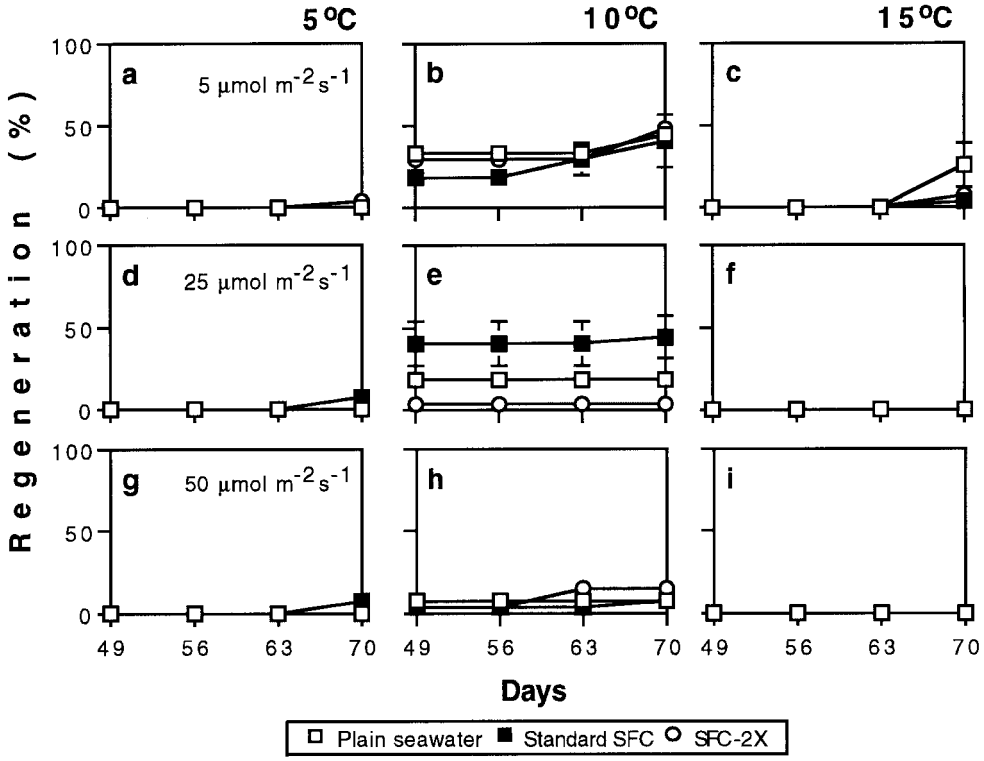

Figure 6. Regeneration of *Gigartina skottsbergii* haptera from Ancud, incubated under combined regimes of temperature, photon flux density and medium strength. Values represent mean and standard error.

the study by Perrone and Felicini (1976) in *Gigartina acicularis.* That study was based on a fluctuating bench temperature (18–25 °C) combined with light up to 1200 lux, and a fixed combination of 20 °C and 1000 lux. Several types of culture media, with different nutrient concentrations, were also included in the study. According to the authors, *G. acicularis* responded better when incubated in full strength culture media or in phosphate-enriched seawater than when grown in plain seawater or seawater enriched with ammonium nitrate only. No different regeneration responses were attributed to changes in light and temperature. As indicated above, however, *G. skottsbergii* seems to respond quite differently. Not only temperature and photon flux density are important determinants in the healing and regeneration success of *G. skottsbergii,* but nutrients appear to trigger opposite responses to those in *G. acicularis*. Indeed, whereas in *G. skottsbergii* full and double strength culture media tend to diminish healing and regeneration success, in *G. acicularis* full strength media, particularly phosphate-reach, seems crucial for a good regeneration performance.

Although information from field data suggests that *Gigartina skottsbergii* is a slow-growing species (Westermeier, pers. obs.), the timing of the various repair steps agreed well with those reported for other algae. In *Eucheuma alvarezii*, for example, the early repair events lead to the formation of an immature cortex by day 12, tissue which completed its development around day 22 (Azanza-Corrales & Dawes, 1989). In *Schottera nicaeensis*, on the other hand, the wound is sealed and regeneration begins after 20–30 days of culture (Perrone & Felicini, 1972). Similarities in the timing do occur even with brown algae like *Sargassum filipendula* C. Agardh, which requires at least 2 months before the cut surface is healed and new uprights are issued from the repaired tissue (Fagerberg & Dawes, 1976). In the case of *Fucus vesiculosus*, at week 6 after the fragments were excised from the frond, fully functional epidermis and cortex were established, issuing uprights which resembled *Fucus* embryos after 12 weeks of culture (Fulcher & McCully, 1969). A pattern that departs from the species indicated above, including *Gigartina skottsbergii*, has been recently reported for *Grateloupia acuminata*,

where fragments from juvenile fronds do not produce new cortical tissue at the cut surface but, instead, issue adventitious filaments which attach to the substratum and produce, afterwards, new fronds similar in morphology to those used to obtain the cuttings (Iima et al., 1995).

In *Gigartina skottsbergii*, regenerated frond initials originated from meristems localized in the new cortex developed at the wound. This direct involvement of the repaired tissue is similar to what has been reported for *Fucus vesiculosus* (Fulcher & McCully, 1969) and *Sargasum filipendula* (Fagerberg & Dawes, 1976), and differs from the regeneration pattern described in other red algae like *Schottera nicaeensis* (Perrone & Felicini, 1972), *Gigartina acicularis* (Perrone & Felicini, 1976), *Eucheuma alvarezii* var. *tambalang* (Azanza-Corrales & Dawes, 1989) and *Gracilaria chilensis* (unpublished). In all these cases, even though wound tissue is known to seal the wounds, new frond initials are issued by the lateral cortex, at some distance from the repaired area.

Due to the cell size and the thickness of the excised fragment, it was not possible to follow the changes in cell content at the cuts in *Gigartina skottsbergii*, as has been described in siphonous green algae (Menzel, 1988). In the latter case, the cytoplasm of the damaged cells contracts, releasing material which coagulates, forming a wound plug. In *G. skottsbergii* this process may be replaced by the large amount of mucilaginous material released at the cut by the exposed tissues. This material, likely cell wall polysaccharides, appear within minutes of the excision and remained in place until the single cell-layer cortex developed.

Polarity was not observed during the regeneration of new frond initials in *Gigartina skottsbergii*. In fact, the issue of new uprights took place along the whole perimeter of the repaired fragments. This is similar to the growth pattern of the plant in the field, where the single and more or less circular blade rests flat on the substratum, increasing its surface by meristematic activity along the border of the thallus. In other algae, however, an extremely marked polarity is established in intact thalli, a condition which is not modified in the excised fragments. This highly conservative polarity results in blade-like thalli from the apical end and rhizoidal filaments, or only a wound scar, from the basal end of the cuts. This process has been experimentally demonstrated in various foliose species including *Schottera nicaeensis* (Perrone & Felicini, 1972), *Gigartina acicularis* (Perrone & Felicini, 1976), *Chondrus crispus* Stack. and *Gracilaria chilensis* Bird, McLachlan et Oliveira (González & Correa, 1996) and *Gelidium lingulatum* Kütz. (unpublished).

In conclusion, control of abiotic factors (i.e. light, temperature and quality of the culture medium) in the laboratory has allowed us to identify the best environment to obtain consistently high regeneration success in *Gigartina skottsbergii*. Healing and regeneration were consistent with most of the available information for other algae, both in the time needed to complete the processes and in the cellular and tissue events involved. These findings are considered as crucial to the success of potential large scale cultivation of *G. skottsbergii*, because it allows circumvention of the 'bottle neck' imposed by the exceedingly short period of time when viable spores can be obtained, usually in small numbers, from wild stocks. Initially, and quite unsuccessfully, most attempts to propagate *G. skottsbergii* were focused in obtaining sporelings. Now, vegetative propagation is possible, not only because the early stages in the process can be successfully manipulated *in vitro* to optimize the production of plantlets, but because those plantlets can develop normally in both tank systems or in the sea, growing at the same rate as wild plants (unpublished). This, together with laboratory manipulation to enhance spore germination and juvenile survival (Correa et al., unpublished data), ensure that an almost continuous supply of inoculum can eventually be made available to farmers.

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