Revista de Biología Marina y Oceanografía
ISSN: 0717-3326
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Universidad de Valparaíso
Chile

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Revista de Biología Marina y Oceanografía, vol. 46, núm. 2, agosto, 2011, pp. 263-268
Universidad de Valparaíso
Viña del Mar, Chile

Revista de Biología Marina y Oceanografía
Vol. 46, Nº2: 263-268, agosto 2011
Research Note

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Efecto del calcio disuelto en la formación de estructuras de adhesión secundaria en diferentes tipos de ramas de *Chondracanthus chamissoi* (Rhodophyta, Gigartinales)

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Abstract.- Development of secondary attachment structures (SAS) was evaluated in apical fragments of pinnules, and basal and lateral branches in the carrageenophyte *Chondracanthus chamissoi*. When no dissolved calcium was added to the culture medium, most basal and lateral branches initiated SAS formation, while most pinnules remained unchanged. Calcium addition greatly increased the proportion of fragments developing SAS, and also increased the stage of development achieved by SAS in all branch types. We suggest that SAS formation by basal branches contributes to a complex attachment system. SAS formation by lateral branches and pinnules may contribute to vegetative propagation following fragmentation of thalli.

Key words: Carrageenophyte, cultivation, edible seaweed, vegetative propagation

Introduction

Chondracanthus chamissoi (C. Agardh) Kützing (Rhodophyta, Gigartinales) is a common seaweed that occurs from Paita, Perú to Ancud, Chile (5-42°S), (Ramírez & Santelices 1991, Hoffmann & Santelices 1997). It grows on hard substrata from the lower intertidal levels to a depth of *ca.* 15 m (Hoffmann & Santelices 1997). It has a triphasic *Polysiphonia*-type life-cycle with isomorphic sporophytes and gametophytes (Ávila *et al.* 2010). Blades are narrow and can attain 50 cm in length, although commonly they are much shorter. Lateral branches of similar morphology to the main axes, and abundant, short and narrow pinnules, grow from the margins of the main axes. Thalli are attached to the substratum by a small basal disc (Hoffmann & Santelices 1997), although a few cylindrical branches usually emerge from the base of the main axes and curve towards the substratum forming a complex attachment system. *Chondracanthus chamissoi* is a resource of commercial importance in Chile. It has been exported as raw material for the extraction of carrageenan, and there is also increasing exploitation for direct human consumption. Basic biological and ecological aspects have been studied, both in laboratory and in the field (González & Meneses 1996, González *et al.* 1997, Vásquez & Vega 2001, Bulboa & Macchiavello 2001, Macchiavello *et al.* 2003, Bulboa *et al.* 2007, 2008, 2010, Fonck *et al.* 2008, Sáez *et al.* 2008, Ávila *et al.* 2010), and attempts have been made to develop management and culturing techniques for this resource (Bulboa *et al.* 2005, Bulboa & Macchiavello 2006).

Vegetative propagation, as an alternative to reproduction via spores, could be used to cultivate this species. In this context, secondary attachment of fragments is common in many seaweeds (Hoffmann 1987, Santelices 1990, Norton 1992). The formation of secondary attachment structures (SAS) by drifting fragments of *C. chamissoi* (Macchiavello *et al.* 2003, Fonck *et al.* 2008, Sáez *et al.* 2008) and other Gigartinaceae (*e.g.*, Pacheco-Ruíz & Zertuche-González 1999, Pacheco-Ruíz *et al*. 2005) has been reported, and these SAS can produce new shoots (Pacheco-Ruíz *et al.* 2005, Sáez *et al.* 2008). Some factors that can potentially affect SAS formation in *C. chamissoi* have been studied determining, for example, that the type of substratum is important, where attachment of fragments was favored on shell gravel when compared to rocky substrata (Fonck *et al.* 2008). No differences in SAS formation were detected between phases of the life-cycle of *C. chamissoi* (Sáez *et al.* 2008), as has been reported in other red algal species (Juanes & Puente 1993).

Other factors may also be important. For example, SAS formation may differ among parts of the thallus (Salinas 1991) or among different types of branches. In the latter case, results may reveal functional differences among the branches, which could be important in the design of cultivation or management methodologies. Also, calcareous substrata have been shown to enhance secondary attachment (Salinas 1991, Juanes & Puente 1993), but it is possible that dissolved calcium added to the culture medium could have a similar effect (Santelices & Varela 1994). In this study we evaluated the effect of three factors that may affect secondary attachment. We quantitatively compared the effect of two types of substrata, as well as the effect of added calcium in the form of dissolved calcium ions in the growth medium, on the formation of SAS in *C. chamissoi*, and we experimentally evaluated this effect using apices obtained from different types of branches. We expected that SAS formation would be most frequent in apices of basal branches, although they also would occur in apices of other branch types. We also expected that the addition of calcium would favor secondary attachment, and that this would occur in both types of substrata.

MATERIALS AND METHODS

Female gametophytic thalli of *Chondracanthus chamissoi* were collected from natural beds in Ramuntcho (36º45'S, 73º11'W) and Lirquén (36º42'S, 72º58'W), Región del Biobío, Chile, between April and July, 2010, and transported to the laboratory for experiments. In all cases, experiments were mounted less than 24 h after collection. In total, four similar experiments were mounted. In all experiments, fragments were placed in aquaria with 1.5 L of microfiltered seawater (0.45 μm) enriched with f/2 medium (Andersen *et al.* 2005) and incubated in culture chambers with a 12:12 photoperiod (light:darkness), at 13 ± 1°C, and 29 ± 5 μmol photons m^{-2} sec^{-1}. Experiments differed in the combination of the following three factors: branch type, addition of calcium and substratum type (Table 1). We used apices from three types of branches: basal branches, pinnules and lateral branches, each cut

Table 1. Combination of experimental factors used in the four experiments to evaluate the formation of secondary attachment structures in *Chondracanthus chamissoi*. The values indicate the number of fragments used in each treatment. Three branch types were used: basal branches (BB), lateral branches (LB), and pinnules (Pi). Calcium (as $CaCl_2$) was added to the culture medium in some of the treatments. The rough sides of ceramics and glass slides were used as substrata / Combinación de factores experimentales usados en los cuatro experimentos para evaluar la formación de estructuras de adhesión secundaria de *Chondracanthus chamissoi*. Los valores son el número de fragmentos usados en cada tratamiento. Tres tipos de ramas fueron usadas: ramas basales (BB), ramas laterales (LB) y pínulas (Pi). Calcio (como $CaCl_2$) fue agregado al medio de cultivo en algunos tratamientos. El lado rugoso de cerámicos y portaobjetos de vidrio fueron usados como sustrato

	Branch type	Calcium addition	Type of substratum	
			Ceramics	Glass slides
Experiment I	BB	no	10	10
	BB	yes	10	10
Experiment II	BB	no	10	-
	BB	yes	30	-
Experiment III	BB	no	5	5
	Pi	no	5	5
	LB	no	10	10
	BB	yes	-	5
	Pi	yes	-	5
	LB	yes	-	10
Experiment IV	BB	no	10	10
	Pi	no	10	10
	LB	no	10	10
	BB	yes	10	10
	Pi	yes	10	10
	LB	yes	10	10

as 1 to 2 cm long fragments that included the tips of branches. Apices were obtained from different individuals. Two concentrations of calcium were used: control treatments had the calcium concentration of seawater (0.4121 g L^{-1} of seawater, Libes 2009), and treatments with increased calcium concentration were obtained by adding $CaCl_2$ to the culture medium (3 g L^{-1}, an increase of 2.6 times the concentration of calcium in seawater). Additionally, as substratum we used fragments of glass microscopy slides and the rough side of ceramic plates. These two types of substrata differ in their chemical composition, roughness and transparency. Algal fragments were placed individually onto pieces of each type of substratum in such a way that the apex of each was in direct contact with the surface of the substratum at a 30 to 60° angle. In all, 260 algal fragments were used (Table 1). Experiments were evaluated every 24 h until Day 4, then every 48 h until Day 8. On each occasion we checked the development stage of any SAS using a dissecting microscope.

The effect of substratum type on SAS formation was compared using a two-dimensional contingency table, pooling the results of all experiments, conducted separately for control and calcium addition treatments. For basal branches, the effect of calcium addition on the proportion of apices that had reached each stage of SAS formation was evaluated by pooling the results of the four experiments for Day 8 for this type of branch, and results were analyzed with a two-dimensional contingency table. The effect of calcium addition on the proportion of apices in each stage of SAS formation for the three types of branches was compared, pooling results for Day 8 only for Experiments III and IV, and results were analyzed with a three-dimensional contingency table.

Results and discussion

Previous observations (Fonseca F & R Otaíza, pers. obs.) allowed us to define three stages in the formation of SAS. Stage I were apices without any development of SAS, *i.e.*, a blunt, rounded apex in which the cuticle was distinguishable but no additional growth could be identified. Stage II were unattached apices with a distinguishable hyaline structure projected from the apex, which only slightly altered the general shape of the apex. Stage III, were apices in which the hyaline structure was clearly distinguishable, and altered the rounded shape of the tip, frequently expanding as an inverted cone. This structure was similar to that described for other *Chondracanthus* species (Pacheco-Ruíz & Zertuche-González 1999, Pacheco-Ruíz *et al.* 2005). Stage III included attached apices, but frequently apices reached this stage without attaching to the substratum. In all three stages, the pigmentation of the cortical cells of the original apex remained distinguishable, contrasting with the hyaline aspect of the developing SAS (Fig. 1).

There were no significant differences in SAS production between fragments growing on different types of substrata, either in the control ($\chi^2 = 0.79$, d.f. = 2, $P > 0.05$) or calcium addition treatments ($\chi^2 = 1.55$, d.f. = 2, $P > 0.05$), therefore results for ceramics and glass slides were pooled. For basal branches, the proportion of apices in the different stages of SAS formation showed significant differences between treatments with and without calcium addition ($\chi^2 = 48.3$, d.f. = 2, $P < 0.001$; Fig. 2). Most basal branch fragments in the control treatment progressed to Stages II and III (50.8 and 30.8%, respectively). In contrast, in the calcium addition treatment, the great majority of the fragments (84.7%) advanced to Stage III.

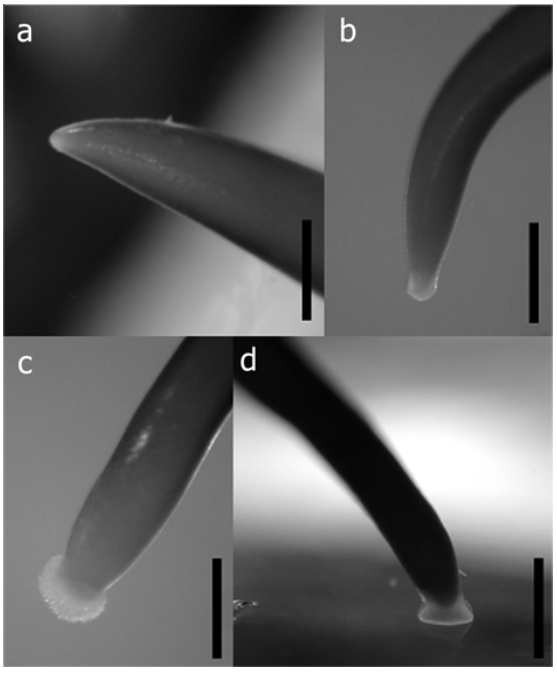

Figure 1. Apices of *Chondracanthus chamissoi* in different stages of development of secondary attachment structures: a) Stage I, b) Stage II, c) Stage III with unattached apex, and d) Stage III with apex attached to the substratum. Scale bars: 1 mm / Ápices de *Chondracanthus chamissoi* en diferentes estados de desarrollo de estructuras de adhesión secundaria: a) Estado I, b) Estado II, c) Estado III con ápice no adherido, y d) Estado III con ápice adherido al sustrato. Barra de escalas: 1 mm

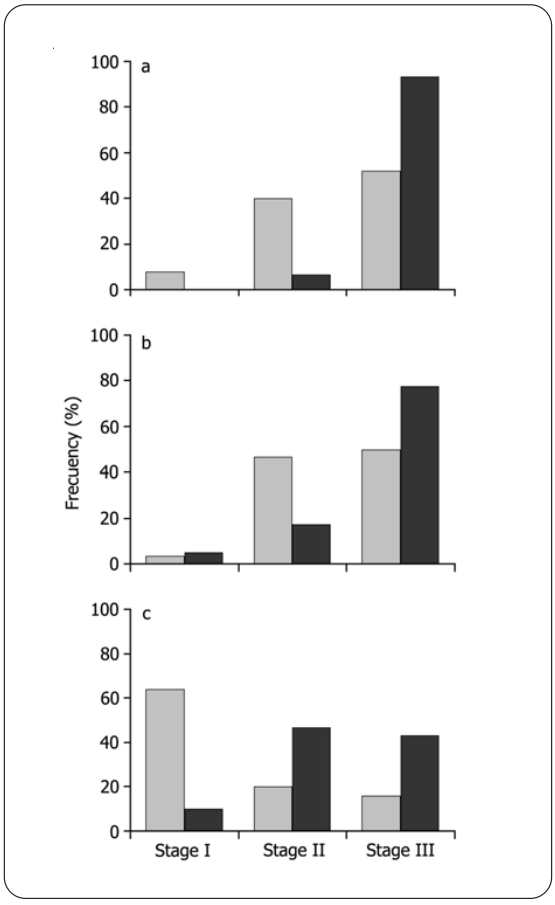

Figure 2. Frequency of fragments of *Chondracanthus chamissoi* **in the three stages of development of secondary attachment structures after eight days of incubation. Fragments of all three types of branches were used: a) basal branches, b) lateral branches, and c) pinnules. Experimental conditions included control (light gray bars) and calcium addition (black bars) treatments. A total of 260 fragments were used** / Frecuencia de fragmentos de *Chondracanthus chamissoi* con estructuras de adhesión secundaria en tres estados de desarrollo al octavo día de incubación. Se incluyeron fragmentos de tres tipos de rama: a) ramas basales, b) ramas laterales, y c) pínulas. Las condiciones experimentales incluyeron tratamientos control (barras gris claro) y adición de calcio (barras negras). En total se ocuparon 260 fragmentos

Significant differences were also found among the three types of branches (χ^2 = 91.9, d.f.= 12, $P < 0.001$). Most lateral branches progressed to Stages II and III (46.7 and 50.0%, respectively) in the control treatment, while the majority (77.5%) progressed to Stage III in the calcium addition treatment. In contrast, most pinnules (64.0%) remained in Stage I in the control treatment, and the great majority advanced to Stages II or III (46.7 and 43.3%, respectively) in the calcium addition treatment. As expected, results for basal branches were very similar to the previous analysis, all progressing to Stages II or III (6.7 and 93.3%, respectively) in the calcium addition treatment. In all, only 12.0% of all apices reaching Stage III were attached to the substrata, and this only occurred in the calcium addition treatment.

This strong response of apices to form SAS when maintained in contact with the substratum, and the enhancement effect of calcium addition supports the idea that *Chondracanthus chamissoi* presents a strategy of vegetative propagation (Macchiavello *et al.* 2003, Fonck *et al.* 2008, Sáez *et al.* 2008), as it has been proposed for other *Chondracanthus* species (Pacheco-Ruíz & Zertuche-González 1999, Pacheco-Ruíz *et al.* 2005). On one hand, it has been shown that *C. chamissoi* undergoes spontaneous fragmentation and detachment of thalli in natural populations (González *et al.* 1997, Vásquez & Vega 2001, Macchiavello *et al.* 2003, Bulboa *et al.* 2005). Characterization of these drifting fragments is lacking and their fate is unknown, so their contribution to population abundance is still to be determined. On the other hand, fragments of all three types of branches produced SAS, but in different proportions. High SAS formation in basal branches, together with the effect of calcium addition in reducing the time required to initiate them, suggests that these branches, which grow towards the substratum, contribute towards consolidating the complex attachment system of individual thalli. In contrast, fragments of lateral branches and pinnules, which grow away from the substratum, but which will readily produce SAS when placed and maintained in contact with the substratum will correspond to vegetative propagules. Other aspects of our results further support this interpretation. First, attachment of fragments occurs in a short period of time. The great majority of the apices responded in eight days (Fig. 2), although only a few completed the process in this period. In other studies of *C. chamissoi*, the time required for attachment ranged from five days to over a month (Macchiavello *et al.* 2003, Bulboa *et al.* 2005, Fonck *et al.* 2008, Sáez *et al.* 2008). This range of values may result from differences in the sampling frequency, but also from differences in the degree of contact of the fragments with the substratum. Attachment success cannot be compared with other studies where either whole plants or branch fragments have been used, but where no indications of the total number of apices making contact with the substratum were given. Second, added dissolved

calcium stimulated attachment. In our experiments, calcium addition resulted not only in an increase in the proportion of fragments developing SAS, but also in the stage of development attained. This was true even for pinnules, which were the least reactive of the three types of branches used. An increase in the number of fragments of *C. chamissoi* attached was also obtained in experiments comparing rocky substrata and shell gravel (Fonck *et al.* 2008). Attachment of other red algal species also increased on calcareous substrata and in calcium addition treatments (*e.g.*, Salinas 1991, Juanes & Puente 1993, Santelices & Varela 1994). In the natural environment, calcareous substrata are not restricted to mollusk shells; the presence of live or dead crustose corallines could also increase calcium concentrations in their immediate vicinity, stimulating re-attachment. This alternative should be experimentally tested. Third, *C. chamissoi* can produce SAS in various types of substrata. In our results, SAS were produced on glass slides and ceramic plates. Other studies have obtained attachment of *C. chamissoi* fragments to rocky substrata, mollusk shells and polypropylene ropes (Macchiavello *et al.* 2003, Bulboa *et al.* 2005, Fonck *et al.* 2008, Sáez *et al.* 2008). Seasonal fragmentation of the blades of *C. chamissoi*, together with re-attachment of drifting fragments and the enhancement effect of calcium can not only have important effects on the population, but this attribute can also be of great importance for restoration techniques of populations or cultivation procedures (Bulboa & Macchiavello 2006, Fonck *et al.* 2008, Sáez *et al.* 2008).

Acknowledgments

The authors are grateful to P. Neill for her very helpful comments and criticism of the manuscript.

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Received 29 March 2011 and accepted 14 April 2011