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Infection process of *Ceramium rubrum* (Rhodophyta, Ceramiales) on the agarophyte *Gracilaria chilensis* (Rhodophyta, Gracilariales)

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Abstract: Epiphytism is a major problem world-wide for the *Gracilaria* mariculture industry that reduces the productivity of commercial farms. Most of the epiphytes associated with *Gracilaria chilensis* belong to the order Ceramiales. Under laboratory conditions we studied the infection process of *Ceramium rubrum* rhizoids on *G. chilensis* collected from a northern Chilean farm. The rhizoid penetration was intercellular. The host cell wall and cortical cells adjacent to the epiphyte appeared compressed and disorganized. The rhizoids presented an extensive dynamic complex endomembrane system, mainly represented by a plentiful endoplasmic reticulum (ER) network, dictyosomes, electron dense material surrounded by ER membranes, membrane bodies and vacuolar sacs with fibrillar material. Two types of cell wall deposition can be suggested: i) one characterized by the direct contact of ER vesicles with the plasmalemma and ii) another represented by the ER contributing material to the cell wall indirectly through vacuolar sacs. This study provides the basis for a better understanding of the infection process involved in the epiphyte-host interaction.

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Keywords: *Ceramium rubrum*; epiphytism; *Gracilaria chilensis*; ultrastructure.

Introduction

Epiphytism on *Gracilaria*, one of the most exploited agarophytes worldwide, has been widely reported both in natural environments and under cultivation conditions (Hayashi et al. 2014).

Farming of *Gracilaria chilensis* has been a commercial activity along the Chilean coast since the mid 1980s (Buschmann et al. 2008) and nowadays, this is the main red seaweed exploited in Latin America (Hayashi et al. 2014). The invasion of epiphytes in farms results in lower levels of productivity through interference with the growth rate of the basiphyte, increased loss of biomass by increased water drag and herbivore attraction, basiphyte detachment promoted by the weight of the epiphyte, a decline in the quality of raw material, as well as increased postharvest cleaning costs (Buschmann and Gomez 1993, González et al. 1993, Buschmann et al. 2008).

Ceramium spp. have been identified as common epiphytes on *Gracilaria* species (Fletcher 1995, Leonardi et al. 2006, Muñoz and Fotedar 2010, Martín et al. 2013) and the dominance of Ceramiales among the epiphytes has been reported for *G. chilensis* farms (Westermeier et al. 1993). In particular *Ceramium rubrum* is one of the main epiphytes growing on *G. chilensis* (Buschmann et al. 1997). The prevalence of epiphytes in northern Chilean farms occurs during most of the year and under favourable environmental conditions, with regrowth of settled individuals producing an increase in the epiphytic load (Buschmann et al. 1997).

Even though there are diverse studies dealing with the epiphytism on *Gracilaria*, most are focused on diversity, distribution and/or phenomenology of the epiphytes (Buschmann and Gomez 1993, González et al. 1993, Fletcher 1995, Buschmann et al. 1997, Leonardi et al. 2006, Muñoz and Fotedar 2010, Martín et al. 2013). Regarding ultrastructural analysis of the interaction epiphyte-host,

there are only a few reports for *Gracilaria* species (Leonardi et al. 2006, Martín et al. 2013). Ceramialean infection of *G. chilensis* and *G. gracilis* is characterized by a deep intercellular penetration of the epiphyte rhizoid and disorganization of the host's cortical cells (Leonardi et al. 2006, Martín et al. 2013). Light microscopy studies of *G. cliftonii* also demonstrated Ceramialean rhizoid penetration into the host (Muñoz and Fotedar 2010). As for the epiphyte infection process, the only comprehensive study of the attachment and penetration of *Ulva lactuca* zooids into *G. tikvahiae* and *G. cornea* was performed by Dawes et al. (2000). Moreover, it is unknown how Ceramialean vegetative thalli and spores settle, attach and grow on *Gracilaria*. The present study aims to analyze the infection process of rhizoids of *C. rubrum* from vegetative thalli and tetraspore germination on *G. chilensis* through laboratory culture experiments.

Materials and methods

Thalli of *Gracilaria chilensis* Bird, McLachland *et* Oliveira and *Ceramium rubrum* (Hudson) C. Agardh were collected in a commercial farm located in Caldera (27°04′S, 70°50′W), northern Chile. Both species were maintained in culture in a seawater filtered medium C (SFC; Correa 1990) at 15°C in 40 μmol photons m^{-2} s^{-1} with a 12-h photoperiod.

Infection assays

Ceramium rubrum fragments (0.1–0.3 cm) were excised from apical branches immersed in sterile seawater and cultivated for a week in 100-ml Erlenmeyer flasks filled with 60 ml of SFC medium until they reached 0.5 cm in length. *Gracilaria chilensis* fragments 1 cm long were inoculated in 100-ml Erlenmeyer flasks filled with 60 ml of non-aerated SFC medium with ten 0.5-cm long vegetative fragments of *C. rubrum*. When rhizoids reached the host surface, fragments of infected tissue were fixed at different stages of the infection process. Control experiments were performed, in which *C. rubrum* fragments were cultured without *G. chilensis* thalli. Infection and control assays were carried out at the same temperature, photoperiod and irradiance as used for culture maintenance.

Thallus fragments of *C. rubrum* tetrasporophytes with mature tetrasporangia were cultivated in SFC medium and spore liberation was induced at 10°C and an 8-h photoperiod. Afterwards, the tetraspores were carefully transferred with a Pasteur pipette into 50-ml flasks with 20 ml of SFC medium containing at least 10 fragments of *G. chilensis* 1 cm long. The flasks were incubated under non-aerated conditions at 15°C and a 12-h photoperiod. The fragments of infected host tissue were fixed at different stages of spore rhizoid development.

Scanning electron microscopy (SEM)

Infected thalli of *G. chilensis* and fragments with settled tetraspores were fixed with 3% glutaraldehyde (Sigma-Aldrich, Saint Louis, MO, USA) buffered with 0.1 M Na-cacodylate buffer (pH 7.4) (Sigma-Aldrich, Saint Louis, MO, USA) containing 0.25 M sucrose. Fixed samples were dehydrated in an increasing acetone series from 10 to 80%, and dried in a critical point dryer. Samples were coated with gold-palladium and examined in a JEOL SM-35 CF scanning electron microscope (JEOL, Akishima, Tokyo, Japan) at the CCT-CONICET Bahía Blanca.

Transmission electron microscopy (TEM)

Fragments of infected tissue were fixed in 3% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) containing 0.25 M sucrose for at least 2 h. Samples were trimmed and transferred to a fresh fixative solution containing 1.5% formaldehyde (Biopack, Buenos Aires, Argentina). Fixation was followed by a series of rinses in cold 0.1 M Na-cacodylate buffer with a gradually decreasing concentration of sucrose, post-fixation for 2 h in 2% OsO_4 (Sigma-Aldrich, Saint Louis, MO, USA) in 0.1 M cacodylate buffer, dehydration in acetone and infiltration in Spurr's resin (Sigma-Aldrich, Saint Louis, MO, USA) over 4 days. Sections were stained with aqueous uranyl acetate (Sigma-Aldrich, Saint Louis, MO, USA) followed by lead citrate (Sigma-Aldrich, Saint Louis, MO, USA) and they were observed in a JEOL 100CX-II transmission electron microscope (JEOL, Akishima, Tokyo, Japan) operated at 80 kV at the CCT-CONICET Bahía Blanca.

Light microscopy

Semithin sections of 0.5 μm from material infiltrated in Spurr's resin were cut with glass knives and stained with toluidine blue for observation under a Carl Zeiss Axiolab microscope with anoptral phase contrast (Jena, Germany).

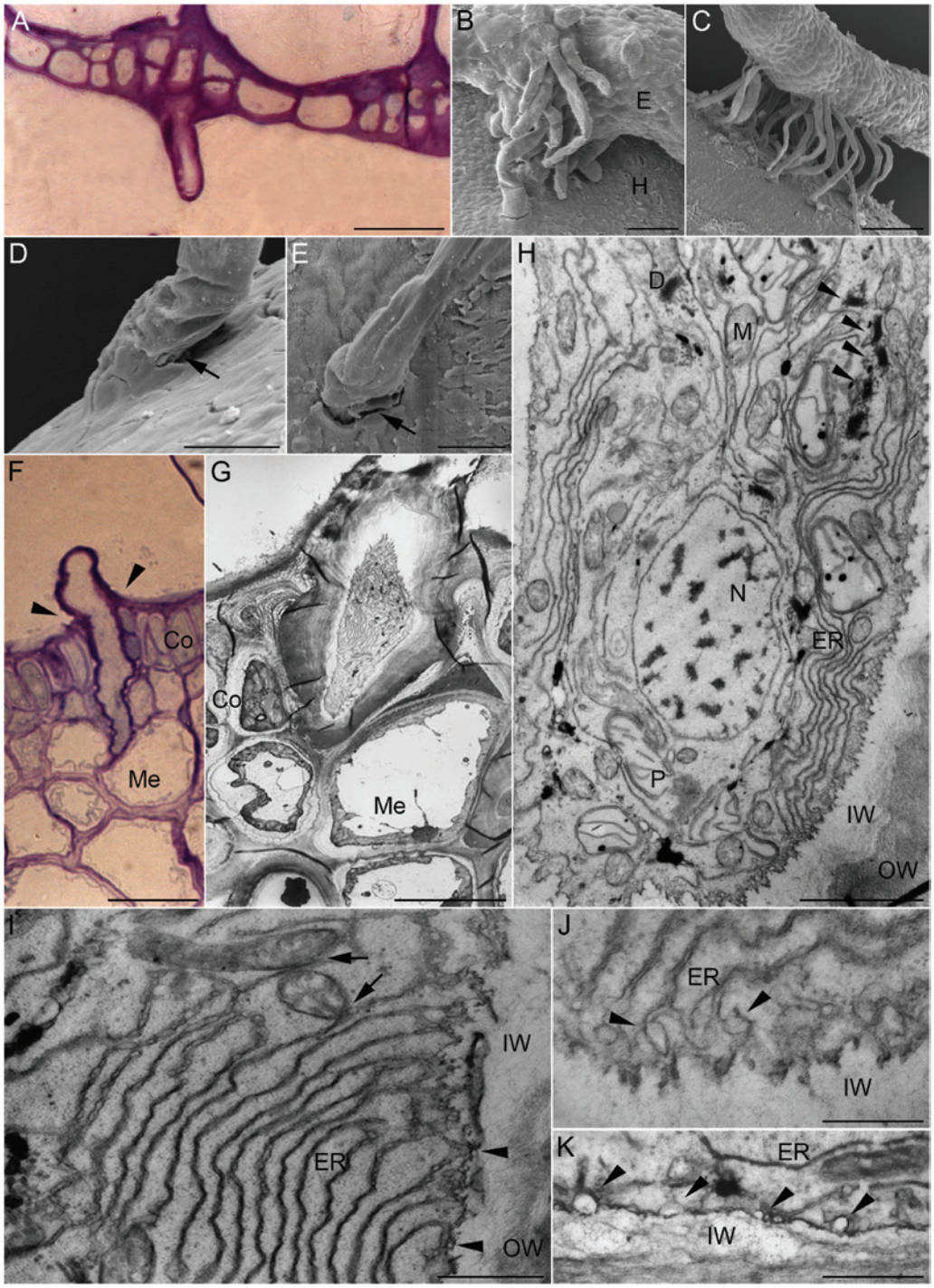

Figure 1: *Ceramium rubrum* vegetative rhizoid development in *Gracilaria chilensis* after 3 days of inoculation (A) and 6 days of infection (B–K). (A) Light micrograph showing rhizoid formation. (B–E) SEM micrographs. (B) Different degrees of rhizoid development reaching the host surface. (C) Numerous rhizoids reaching host surface. (D) Detail of rhizoid penetrating the host cuticle. Note the cuticle partially covering the rhizoid surface (arrow). (E) Detail of hole in host with sharp edges originating from the rhizoid penetration (arrow). (F) Light micrograph of rhizoid penetration reaching the first layer of medullary cells. Note the removal of the cuticle on host surface (arrowheads). (G–K) TEM micrographs. (G) General view of a longitudinally sectioned rhizoid. (H) Portion of cytoplasm of rhizoid tip, showing the organelle distribution. Arrowheads indicate the dictyosome- mitochondria association. (I) Detail of ER cisternae in contact with the plasmalemma (arrowheads) and close proximity of ER and mitochondria (arrows). (J) Detail of vesicles originating from ER dilations located close to the plasmalemma (arrowheads). (K) Detail of ER vesicles fused with the plasmalemma and releasing their content into the inner cell wall (arrowheads). Co, Cortex; D, dictyosome; E, epiphyte; ER, endoplasmic reticulum; H, host; IW, inner cell wall; M, mitochondrion; Me, medulla; N, nucleus; OW, outer cell wall; P, proplastid. Scale bars=20 μm (A, D, F), 50 μm (B, E), 200 μm (C), 10 μm (G), 2 μm (H), 1 μm (I, J, K).

Results

Fragments of *Ceramium rubrum* close to *Gracilaria chilensis* thalli developed adventitious rhizoids from evaginations of cortical cells after 3 days of inoculation (Figure 1A). In contrast, control experiments showed no rhizoid formation in *C. rubrum* fragments when they were grown without *G. chilensis* thalli. To study rhizoid attachment, four infection stages were examined at 6, 14, 21 and 30 days. Scanning electron microscopy observations of the host-epiphyte interface on day 6 of infection revealed either numerous rhizoids with different degrees of development (Figure 1B) or a profusion of many rhizoids reaching the host surface (Figure 1C). Areas around the attachment site were characterized either by the cuticle peeling (Figure 1D) or by the presence of an entrance hole with sharp edges originating from slight cracks in the cuticle (Figure 1E).

Gracilaria chilensis cortical cells showed no change compared with healthy thalli and consisted of 1–2 cell layers, with the outermost cells elongated anticlinally and the inner cells more isodiametric in shape (Figure 1F). At this stage, the host cuticle enclosed the intrusive rhizoid, which penetrated between the cells, breaking through into the cortex in a sinuous way up to the first layer of medullary cells (Figure 1F). A compression of cortical cells in contact with the rhizoid at the attachment site was observed, whereas medullary cells that were adjacent to the rhizoid remained healthy (Figure 1G). The most distinctive feature of the rhizoids was the large number of endoplasmic reticulum (ER) cisternae, which were arranged approximately parallel to each other (Figure 1H). Numerous dictyosomes with electron dense cisternae and vesicles, some mitochondria close to the cis-face dictyosomal cisternae, and plastids were also present in the rhizoids (Figure 1H). Rhizoids had a cell wall composed of a thin lax inner layer and a thick dense outer layer. The plasmalemma adopted a convoluted outline (Figure 1H, I). The close proximity of the ER and mitochondria as well as an ER network adjacent to and in contact with the plasmalemma were evident (Figure 1I). Many vesicles with fibrillar material, originating from ER dilations, were present near the plasmalemma (Figure 1J). Similar vesicles were also observed in contact with the plasmalemma (Figure 1K). The content of these vesicles was identical in appearance to the extracellular material deposited in the inner cell wall (Figure 1K).

On day 14 of infection, evidence of cell alteration appeared in the host cortex, consisting of hyperplasia, compressed cell content and disruption of cortical layers (Figure 2A). The hyperplasia at the site of attachment consisted of 2–3 cortical cell layers, compared with 1–2 cortical cell layers in the non-infected areas, as seen with light microscopy (Figure 2A). TEM observations showed changes in the host cell wall around the penetrating rhizoid represented mostly by microfibril compression (Figure 2B). Cellular disorganization of the cortical cell layer was observed; it appeared mainly as compression of the cortical cells at the area of attachment. These cells had a disorganized and compacted cytoplasm (Figure 2B). At this stage, the proximity between the cis-face of the dictyosomes and the mitochondria in the rhizoid tip remained. An irregular outline due to the waving of the plasmalemma was noticed (Figure 2C). Besides, relationships between the ER and the trans-face of the dictyosomes and between the ER and the mitochondria were also observed (Figure 2C). Rhizoids at this stage exhibited a kind of compartmentalization with large stacks of numerous parallel ER cisternae occupying a large region of the tip (Figure 2D). As infection proceeded, the cisternal spaces of the ER filled with electron dense material (Figure 2E). From this material, small membrane bodies (MB) that remained continuous with the ER cisternae were formed (Figure 2E, F). The association between the ER and the cis-face of the dictyosomes was also noticed (Figure 2F). In addition, vacuolar sacs (VS) with fibrillar content were seen originated from the ER. In some sections, the sacs appeared discontinuous and in contact with the plasmalemma (Figure 2G). Vacuolar sac contents were similar in appearance to the extracellular material in the wall. The sacs finally fused with the plasmalemma and their contents were confluent with the cell wall matrix (Figure 2H). This material was observed with a layered distribution at the wall around the rhizoid tip, acquiring a fibrillar aspect and becoming a part of the rhizoid's inner wall that increased its thickness considerably (Figure 2B, D, H, I).

On day 21 of infection, light micrographs showed serious damage in the *G. chilensis* cortex (Figure 3A). Host cortical cells, adjacent to the rhizoid appeared highly compressed and, in some of them, only the floridean starch granules could be distinguished (Figure 3B). Adjacent medullary cells, however, appeared to be healthy (Figure 3A, B). The rhizoids had a thick wall and high vacuolisation (Figure 3B). The proximity of mitochondria to both dictyosomes and ER remained as it was in the previous stages (Figure 3C). Well-developed proplastids with numerous plastoglobuli were also present (Figure 3C). A network of fragmented ER close to, and in contact with, the plasmalemma was observed (Figure 3D).

On day 30 of infection, rhizoids showed no significant ultrastructural changes relative to the previous stage, except for the increased cell wall thickness. In the

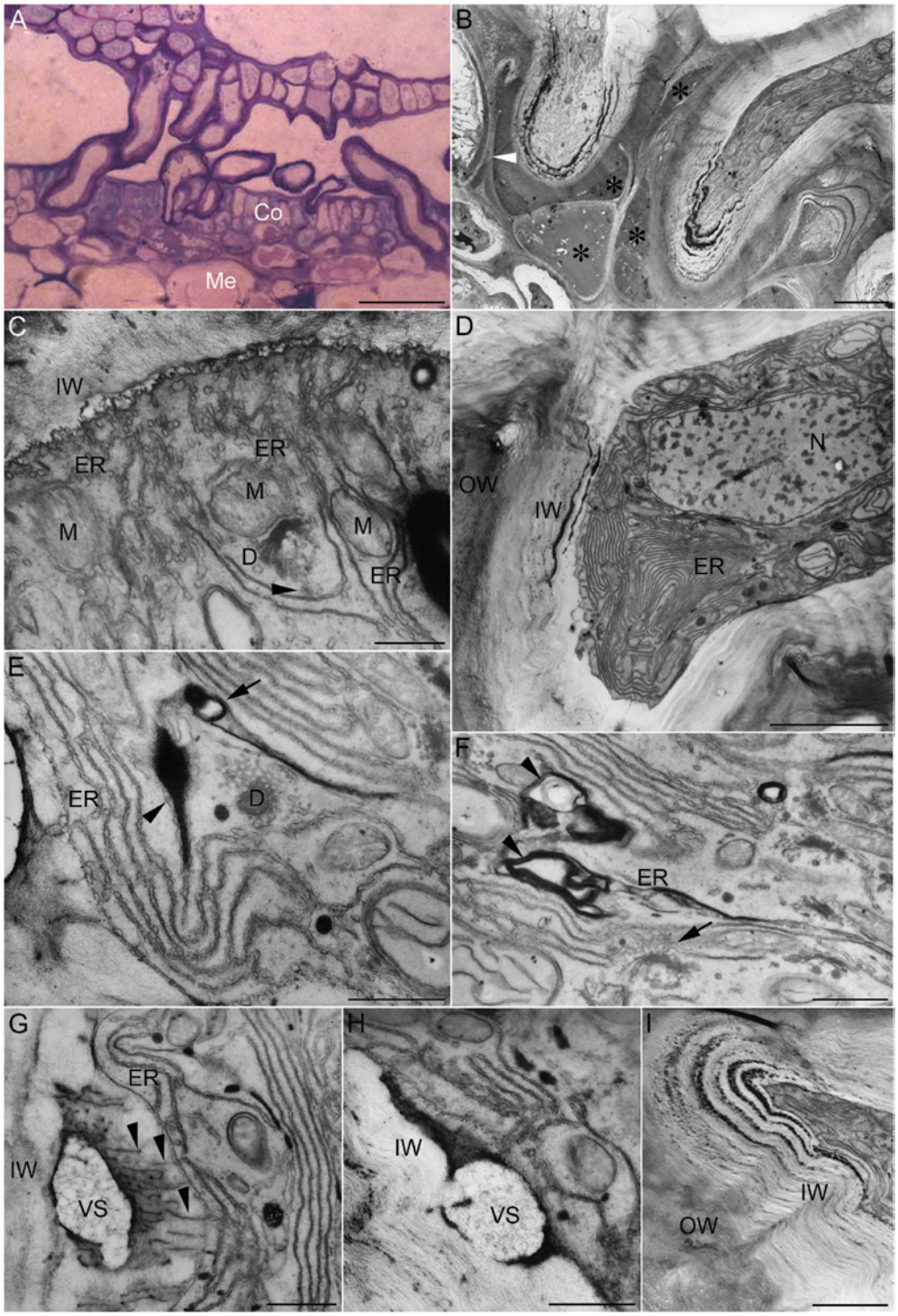

Figure 2: *Ceramium rubrum* vegetative rhizoid development in *Gracilaria chilensis* after 14 days of infection. (A) Light micrograph of rhizoid penetration showing the hyperplasia at the cortex. (B–J) TEM micrographs. (B) Penetration of two rhizoids and cellular disorganization and compression of the host cortex (asterisks). Note also the cell wall compression (arrowhead). (C) General view of the association between the cis-face of a dictyosome and a mitochondrion, and between the trans-face of a dictyosome and ER (arrowhead), and of the close proximity of ER and mitochondria. Note the wavy plasmalemma. (D) Parallel ER cisternae occupying distal portion of rhizoid tip. (E) ER in association with dictyosome cisternae, dilated ER cisternae with electron dense material (arrowhead) and an incipient MB (arrow). (F) MB originating from ER cisternae (arrowheads) and association between ER and cis-face dictyosome (arrow). (G) Vacuolar sac originated from the ER (arrowheads) and in contact with the plasmalemma. (H) VS secreting wall material to the inner layer. (I) Rhizoid tip with layers of electron dense material in the inner wall. Co, Cortex; MB, membrane body; D, dictyosome; ER, endoplasmic reticulum; IW, inner cell wall; M, mitochondrion; Me, medulla; N, nucleus; OW, outer cell wall; VS, vacuolar sac. Scale bars=20 μm (A), 5 μm (B, D), 1 μm (C, E–H), 2 μm (I).

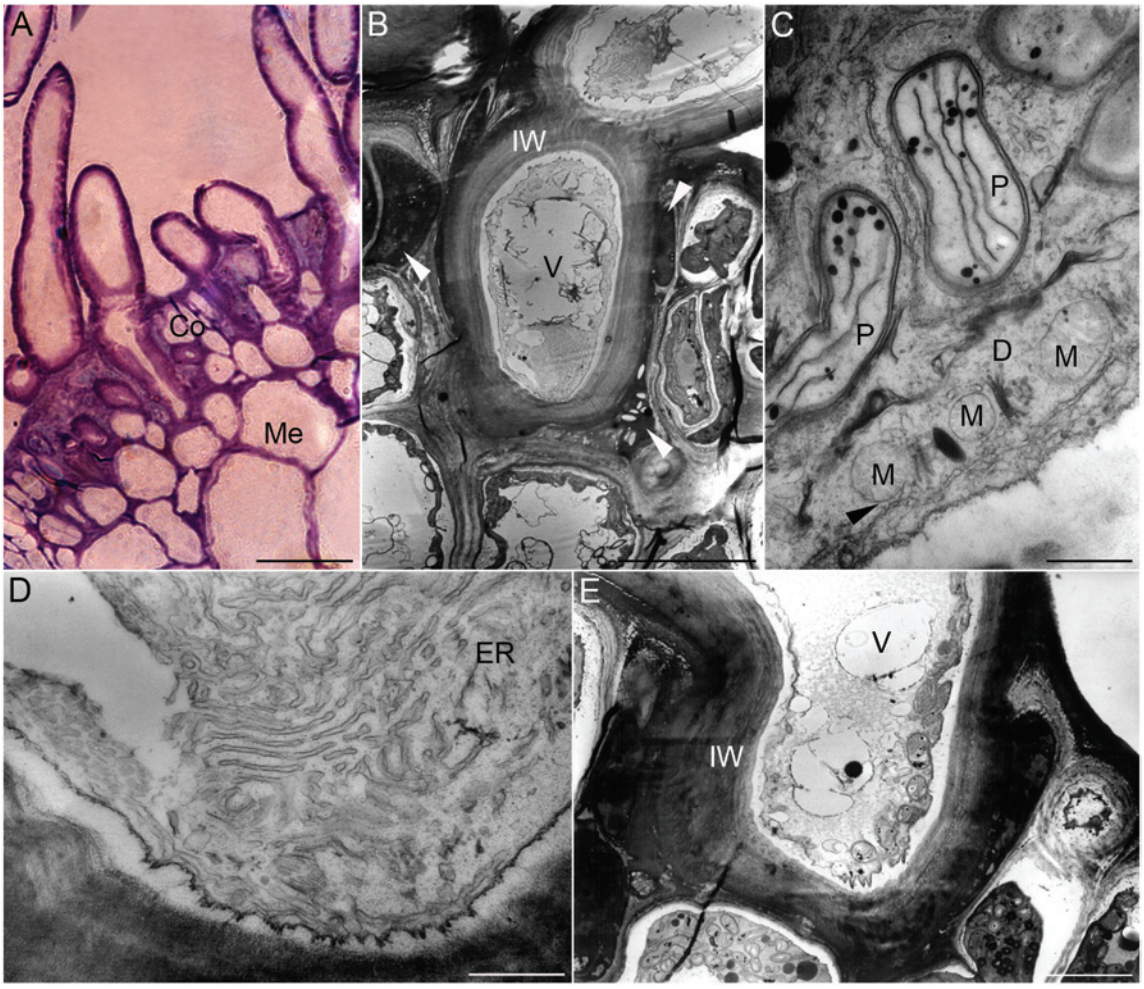

Figure 3: *Ceramium rubrum* vegetative rhizoid development in *Gracilaria chilensis* after 21 (A–D) and 30 (E) days of infection. (A) Light micrograph of rhizoid penetration showing severe damage to cortex. (B–E) TEM micrographs. (B) Vacuolated rhizoid with a thick wall. Note host cortical cells highly reduced and compressed around rhizoid penetration (arrowheads). Floridean starch granules can be distinguished. (C) Detail of well-developed proplastids, the association between dictyosomes and mitochondria, and between ER and mitochondria (arrowhead). (D) Detail of a network of fragmented ER close to and in contact with the plasmalemma. (E) TEM micrograph of a highly vacuolated rhizoid with a thick wall. Co, Cortex; D, dictyosome; ER, endoplasmic reticulum; IW, inner cell wall; M, mitochondrion; Me, medulla; P, proplastid; V, vacuole. Scale bars=20 μm (A), 5 μm (B, E), 1 μm (C, D).

same way no changes were observed in the host anatomy (Figure 3E).

Regarding the tetraspore infection process, mature tetrasporophytes of *C. rubrum* released spores after 5 days of culture. Tetraspores surrounded by hyaline mucilage settled on *G. chilensis* thalli (Figure 4A). After attachment, they showed a pattern of bipolar germination developing an apical cell and an elongated colourless primary basal rhizoid (Figure 4B). In the 3-day-old germlings, the rhizoid was partially surrounded by the host cuticle and rhizoid penetration into the first layer of host cortex was seen (Figure 4C). The cell wall and the cytoplasm of the *Gracilaria* cortical cells, which were located around the rhizoid, appeared to be compressed by its penetration.

In 5-day-old germlings, rhizoids grew intrusively through the second layer of cortical cells reaching the first layer of medullary cells (Figure 4D). The rhizoidal tip was highly vacuolated in the middle portion and numerous floridean starch granules were seen (Figure 4D). The cytoplasm had an ER network adjacent to and in contact with the plasmalemma (Figure 4E, F, G). In addition, electron dense bodies originating from the ER cisternae were present (Figure 4E, F, H). Numerous proplastids with an elongated shape and a poorly developed thylakoidal system were also observed in this portion of the rhizoid (Figure 4F). Dictyosomes associated both with ER through their trans-face and with mitochondria through their cis-face were present around the rhizoid tip (Figure 4G).

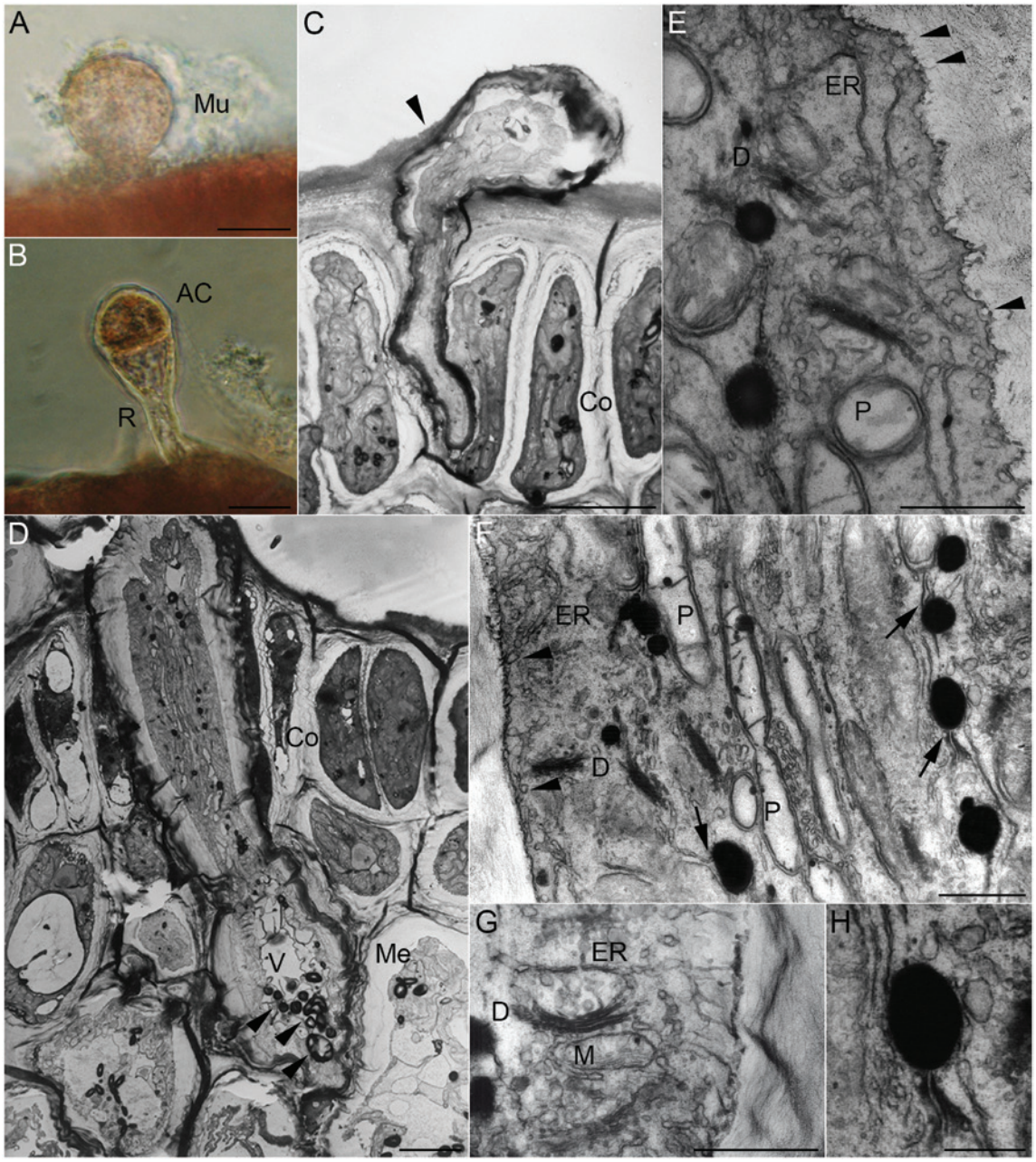

Figure 4: *Ceramium rubrum* tetraspore development in *Gracilaria chilensis*. (A–B) Light micrographs of tetraspore settlement and germination, respectively. (C–H) TEM micrographs. (C) 3-day-old rhizoid penetrating first layer of cortical cells; arrowhead shows rhizoid surrounded by host cuticle. (D–H) Five-day old germlings. (D) Rhizoid penetrating second layer of cortical cells and reaching first layer of medullary cells; arrowheads indicate numerous floridean starch granules. (E) ER network adjacent to and in contact with the plasmalemma (arrowheads). (F) Electron dense bodies originating from ER (arrows). ER network adjacent to and in contact with the plasmalemma (arrowheads). Note the elongated proplastids. (G) View of rhizoid with a dictyosome associated with ER through its trans-face and a mitochondrion through its cis-face. (H) Detail of electron dense body originating from ER. AC, Apical cell; C, chloroplast; Co, cortex; D, dictyosome; ER, endoplasmic reticulum; M, mitochondrion; Me, medulla; Mu, mucilage; P, proplastid; R, rhizoid; V, vacuole. Scale bars=10 μm (A), 20 μm (B), 5 μm (C, D), 1 μm (E–G), 0.5 μm (H).

Discussion

The interaction between *Gracilaria chilensis* and *Ceramium rubrum* is defined as one of the most aggressive infections based on the anatomical relationships between the epiphyte and the host. This interaction, which is described as type V infection by Leonardi et al. (2006), is characterized by a deep epiphyte penetration into the cortex up to the

medullary tissue, with destruction of the host's cells in the area around the infection. Once *C. rubrum* was in contact with thalli of *G. chilensis*, numerous rhizoids were developed from vegetative thalli and tetraspores. When fragments of *C. rubrum* were cultivated without *G. chilensis* thalli, however, they did not develop rhizoids. This is not surprising as *G. chilensis* releases extracellular polysaccharides that stimulate the attachment of epiphytic algae, providing a signal and a site for the epiphytes (Santelices and Varela 1993).

The penetration by vegetative rhizoids was similar to that described for germling rhizoids with respect to both the anatomical changes induced by the epiphyte in the host, and the endomembrane system involved in the epiphyte cell wall formation. The vegetative rhizoid penetration process was also comparable in the timing of germling rhizoid infection. Hence, the stages recorded for vegetative rhizoid penetration were correlated with those for tetraspore rhizoid penetration. In both cases the penetration occurred very quickly since rhizoids reached the medulla in less than a week.

The presence of sharp edges observed with SEM at the entrance hole of *G. chilensis* surface during rhizoid penetration would suggest an enzymatic degradation of the cuticle, rather than a mechanical rupture. Correa and McLachlan (1994) described a similar penetration pattern in *Chondrus crispus* for the green endophyte *Acrochaete operculata*, and other authors have also suggested that enzymatic digestion of host cell walls accompanies epiphyte/parasite penetration in different red algal associations (Nonomura 1979, Goff 1982). On the other hand, González et al. (1993) associated rhizoid penetration by *Ceramium* spp. with the weakening of *G. chilensis* thalli. The damage became exacerbated further with the attempt to remove epiphytes at commercial farms (Ugarte and Santelices 1992) and with herbivore attraction caused by epiphytes (Kuschel and Buschmann 1991). In the same way, the presence of cracks on the cuticle caused by epiphytism was recorded in the red alga *Kappaphycus alvarezii* associated with a weakening of the host thalli, making them vulnerable to breakage and bacterial attack (Vairapan 2006).

The penetration of rhizoids of *C. rubrum* into the host was always intercellular, accomplished mainly by mechanical means. The significant compression of the cell wall microfibrils exactly in the vicinity of penetrating rhizoids supports this view. Even though the rhizoids were found in close contact with host cells, no secondary pit connections occurred between *G. chilensis* and *C. rubrum*, as occurs in parasitic red algae (Goff and Zuccarello 1994). Rhizoids caused damage to host cortical cells closer to the epiphyte through a severe cytoplasm compression that caused cell disorganization. The anatomy of infected *G. chilensis* cells was compared with healthy thalli and also with those of non-infected thalli described by Bird et al. (1986), indicating cortex hyperplasia. The number of dead host cortical cells increased at an advanced infection stage; however, the medullary cells remained healthy. The same pattern was observed for epiphytism of *C. rubrum* on *G. chilensis* growing in the field (Leonardi et al. 2006). The plasmalemma undulation described in *G. chilensis* cortical cells was also observed in *G. gracilis* infected by the ceramialean *Neosiphonia harveyi* as a consequence of the mechanical action of rhizoid penetration (Martín et al. 2013).

The intrusive rhizoid of *C. rubrum* possessed an extensive dynamic complex endomembrane system, mainly represented by a plentiful ER network, dictyosomes, membrane bodies (MB) and specialized vacuoles called sacs, presumably involved in the active synthesis of cell wall materials. Even though MB and sacs were not observed in germling rhizoids on the fifth day of infection, the presence of electron dense bodies originated from the ER cisternae in association with dictyosome trans-faces represents unambiguous evidence of the same endomembrane dynamics as described for vegetative rhizoids. A similar endomembrane system to the one reported in this study has previously been reported in trichoblast cell wall formation in the ceramialean *Osmundea* (Delivopoulos 2002). The participation of both the dictyosomes and the VS in the synthesis and secretion of wall matrix materials of red algae was corroborated a long time ago (Tsekos et al. 1993 and cited references). Moreover, ER vesicles contributing to cell wall formation have also been proposed in the red alga *Rhodymenia* (Delivopoulos 2004a). In addition, ER direct connections with the plasmalemma have already been implicated in the production and secretion of carbohydrate-rich products in other red algal cells (Pueschel 1990). In actively growing rhizoidal cells of parasitic red algae, however, ER cisternae connected to the plasmalemma were not related to wall synthesis, but they are thought to translocate absorbed nutrients (Goff 1979, 1982).

Endoplasmic reticulum cisternal spaces filled with electron dense material like those described in *C. rubrum* were also observed during carposporogenesis of the red alga *Callophyllis linearis* (Delivopoulos 2004b). On the other hand, Rawlence and Taylor (1972) showed electron dense material in the peripheral cytoplasm of rhizoids of *Polysiphonia lanosa* growing into *Ascophyllum nodosum* at the cytoplasm/wall interface, indicating a discharge of material related to rhizoid expansion. The participation of ER in the formation of VS was also observed during tetrasporogenesis in the red alga *Palmaria palmata* (Pueschel

1979). Furthermore, as indicated above, there is evidence that these sacs are involved in the synthesis and secretion of some cell wall matrix materials in vegetative cells and also in reproductive cells of other red algae, such as *Chondria tenuissima*, *Ceramium diaphanum*, *Gigartina teedii* and *Spermothamnion johannis* (Tsekos 1996). Moreover, MB originated from ER and, in turn, VS originated from MB have previously been described in the red algae *Faucheocolax attenuata* and *Osmundea spectabilis* (Delivopoulos and Krugens 1984, Delivopoulos 2002). On the other hand, both the dictyosomes associated with ER through their trans-face and the presence of sacs described in *C. rubrum* agree with the sequence of organelles involved in plant vacuole formation (Robinson 1984, Staehelin 1997, Marty 1999).

In short, during rhizoid growth, two types of cell wall deposition can be suggested: i) one characterized by the direct contact of ER vesicles with the plasmalemma, which release their contents to the cell wall; and ii) another represented by the ER contributing material to the cell wall indirectly through the vacuolar sacs. These two types of ER behaviour may contribute different precursors to the cell wall; however, cytochemical studies are necessary in order to corroborate these ultrastructural observations.

The close proximity between mitochondria and the cis-face of the dictyosomes was observed during all developmental stages of *Ceramium* rhizoids. According to Pueschel (1990), all the Florideophyceae critically examined have the same association; however, its function is still unclear. In addition, the close proximity of ER and mitochondria were observed during all infection stages. These associations might be related to bioenergetics and survival functions of the cells (Giorgia et al. 2009).

Most of the epiphytes associated with *G. chilensis* belong to the order Ceramiales, which are known for their seasonal pattern and a short simple life cycle dominated by an asexual stage; these are regarded as two opportunistic strategies for mass colonization and invasion success (Westermeier et al. 1993). Moreover, thin red filamentous epiphytic algae, such as *Ceramium* spp. with high surface-to-volume ratios, show quick nutrient uptake, high reproductive capacity and considerable competitive advantage over the cultivated species; therefore, they are universally recognized as troublesome organisms (González et al. 1993).

This study provides the basis for a better understanding of the infection process involved in the *C. rubrum*-*G. chilensis* interaction.

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